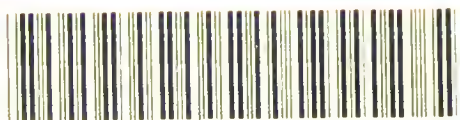




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WORKS BY J. L. W. THUDICHUM, M.D.

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ANNALS  
OF  
CHEMICAL MEDICINE



INCLUDING

THE APPLICATION OF CHEMISTRY TO PHYSIOLOGY,  
PATHOLOGY, THERAPEUTICS, PHARMACY,  
TOXICOLOGY, AND HYGIENE

VOL. I.

1875

EDITED BY

J. L. W. THUDICHUM, M.D.

LONDON  
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1879

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## PREFACE.

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I HAVE the honour of laying before the Medical Profession and the general scientific public the first volume of a new periodical, the principal object of which will be the advancement of the sciences named on its title by means more especially of the chemical method. The several means by which it is hoped to contribute to the attainment of this object will be the publication of original researches to be carried out in physiological, pathological, chemical, and pharmaceutical laboratories in Great Britain and abroad, and the communication and diffusion of the results of the progress of chemistry as far as it relates to medical objects which has been made on fields, and communicated through channels, not commonly accessible to medical readers. In this endeavour it had to be borne in mind that some of these results might in their original form be too technical to be readily appreciated, and might therefore admit of some interpretation and adaptation to particular wants. On the other hand it was felt that the lasting value of the publication would be increased by a thoroughly practical treatment of the principal scientific questions of our time, including the communication of technical details which may be necessary to enable the reader to control, or the student and inquirer to repeat and extend, the operations of which the data furnished are the result.

Medical science is at present more particularly interested in the solution of the problems concerning the nature of the causes of infectious diseases, and the nature of the processes of diseases engendered in the organism by these causes. These latter act after the manner of ferments, and while some of them are undoubtedly organised self-reproducing parasitical beings, others are supposed to be unorganised or shapeless, and in this respect to resemble the normal ferments of particular organs of living beings. The proximate and final effects of both kinds of disease-causes upon the body are always massively chemical; the ferments decompose materials of the body into substances which either engender increased consumption of oxygen and excessive production of organic heat, or act as poisons upon the nervous and muscular systems; some are eliminated, causing loss of power and material, others are left as useless or hurtful hindrances in organs and tissues. All infectious diseases have therefore at one period or another chemical results, and these are amongst the principal objects of the inquiries proposed to be expounded in these pages.

Although physiology has its own rights as a science quite independently of practical applications, its development thus far is, and in the future probably will be mainly due to its obvious connection with the healing art. It is in this relation that we shall mainly endeavour to illustrate its progress, but without excluding concise philosophical views or instructive generalisations extending to fields of natural history.

The chemical processes of disease can only be measured with the aid of physiological methods, but the attempt to unfold their quality requires also the application of a kind of chemical method which goes beyond that ordinarily employed by natural historians. The application of this method promises to be of great use in the study of disease, as it has taught us already that several zymotic processes have great analogy to forced decompositions of organoplastic substances by merely chemical agencies.



It is intended to give to the pathological experiment due scope, even though it should in some cases not directly or at once lead to any chemical development. The pathic process shows the chemical share of its composition when it has to be counteracted, be it by prevention or antidosis. Even the most modern treatment of wounds relies to a large extent upon chemical agents, and what is commonly termed antiseptic, might in a wider and perhaps better sense be termed chemical surgery. The practice of the healing art in all its branches is therefore literally interwoven with chemical principles and problems. To assert these principles, and aid in the solution of these problems, and subsidiarily, to effect the union in a focus of data which by distribution would be weak and inert, are parts of a programme which it is hoped may be found not unworthy of the kind attention and generous support of the Medical Profession.

While hoping to merit this countenance mainly by presenting the researches and observations of others, whose results I shall endeavour to give with due completeness and perspicuity, I shall venture to direct attention from time to time to work more particularly my own, and that in parts where progress has been made possible only by the support and countenance which the Lords of the Privy Council and the Local Government Board have given to my researches by connecting them with the Medical Department of their service.

During a long series of years the results of my work on the field of chemical medicine have been published in various repositories of original information, such as 'Reports of the Medical Officer of the Privy Council' 1866 to 1876; 'Reports of the Medical Officer of the Local Government Board' 1876 to 1879; 'Journal of the Chemical Society' 1860 to 1876; 'British Medical Journal' 1864; 'Transactions of the Medical Society of London'; Erdmann's 'Journal of Practical Chemistry'; Liebig's 'Annals of Chemistry'; 'Proceedings of the Royal Society'; 'Moniteur Scientifique'; 'Proceedings of the Royal Bavarian

Academy'; 'The Lancet,' etc. The fragmentary mode of publication, with the circumstance that I have never hitherto published my results in a collected form, has probably been of disadvantage to me with many readers. And it has facilitated a very unworthy use of my work by certain authors; who being fully aware of the work in the places of original publication, have observed complete silence regarding it, or have sought to set it aside with some frivolous objections, while perhaps at the same time appropriating more or less of its fruits. Both in justice to myself, and in regard for the honest literary interests of science, I have at last felt myself obliged to make distinct public reference to some such proceedings, and the protests which I have had to make in some glaring cases will be recorded in Article XXIII. of this volume.

The explanation of the case is not far to seek. There are professed physiologists in this country and abroad, who though very imperfectly acquainted with either the theory or the practice of chemistry, have nevertheless affected to speak as persons having well-founded convictions on the subject: with their pretended convictions, however, resting on no better basis than the haphazard opinion (*obiter dicta*) of some professed chemist or other, or the conceited and sensational papers which, in chemistry as elsewhere, are written for mere claptrap and without research. I could quote instances by the score, where physiologists acting on that system have reproduced as their own the errors of others while omitting the better knowledge which they ought to have given to their readers.

Among the professed chemists there has sometimes been equal absence of knowledge, but, in such cases, as a rule, more discretion. The acquaintance of the chemists with the physiological, pathological, and medical applications of chemistry has often been most slender; but the chemists have appreciated, better than physiologists could, that discussion in chemistry is eventually an affair of THE BALANCE, and they have known that on such discussion they could not openly enter without having first

made actual exact researches of their own. Such researches,—researches which do not pay—the gentlemen to whom I refer have not undertaken. Some, nevertheless, men of externally high position, have persistently done all they could, sometimes by silence, sometimes by innuendo, sometimes by obstruction, to thwart the endeavours which others have made to advance medical science by original chemical researches. These men, however challenged, have avoided the light of publicity, and have thus impressed upon their action a character of intrigue and unfair rivalry. With the progress of time this poor policy has reacted upon its authors, whose universal incompetence to judge in medical chemistry is now either notorious or of easy demonstration.

While I have thus been obliged to forewarn the Medical Profession and the general scientific public regarding the origin of sundry kinds of opposition which the present undertaking may perchance have to face, I all the more hope that the true scientific chemist may derive from the perusal of the ‘Annals of Chemical Medicine’ an assured experience to this effect, namely—that the accurate methods of which he holds the formula in his analytical training, and in the talisman of the atomic theory, are essential in medical no less than in abstract chemistry, and that inaccurate methods must not be propounded to the Faculty under the excuse, which hardly admits of serious examination, that they are ‘good enough for clinical purposes.’

In conclusion I venture to hope that the ‘Annals of Chemical Medicine’ will be useful to several classes of readers. To the medical practitioner they will afford information on the chemical aspects of the most important questions of the science of which he represents the executive authority; collaterally they will afford information on the most trustworthy methods of diagnosing morbid chemical conditions, and on the significance of these conditions with regard to prognosis and treatment. To the scientific inquirer they will present in turns all the latest data from which he will have to start as a basis, if seeking to

enlarge the present information on any one of the subjects within their range.

All contributions of original information, be such the result of observation or experiment, will be as welcome as deductive or inductive meditations, provided only they are based upon the data and principles of actual science. A predilection for a maximum of fact and a minimum of speculation does not exclude the sense of the attractions of theory, in its true meaning, as the very flower and fruit of the slow growth of the body of data. Experience, however, shows that no interpretation of natural phenomena has any lasting value, unless all the essential factors entering into their production are ascertained and appreciated. The accumulation of these data in a form readily accessible to all interested in their use will therefore promote the philosophical development of our science much more than premature generalisations.

With the aid of the list of articles at the beginning, and the alphabetical indices, one of matters, the other of authors, at the end of the volume, the inquirer after information will be fully able to find any general subject or any matter of detail. To facilitate the use of any new terms and symbols, which may be found convenient abbreviations of ideas, I have added a page for their interpretation and definition. The same page will state some synonyms, and thus aid in obviating a confusion which should gradually be abated by the adoption of a uniform nomenclature.

I believe the chemical method to possess vast capabilities for enlarging the domain of pathology and medicine, and for advancing the art of healing disease; and I trust that all who share that hope will aid in its realisation either as contributors, as readers, or as friends.

J. L. W. THUDICHUM, M.D.



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# ANNALS OF CHEMICAL MEDICINE.

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## I.

### *THE CHEMICAL CONSTITUTION OF THE ORGANO-PLASTIC SUBSTANCES CONSIDERED WITH THE AID OF THE HYPOTHESIS OF THEIR AMYLONIDE NATURE.*

THE material which forms the solid and rigid parts of the structure of plants is *cellulin*. This name has been selected because microscopical science has found that the simplest shaped elements of vegetable structure are cells, and that all more complicated structures arise out of aggregated cells by processes of peculiar union and fusion; that the rigid woody part of cells is also the principal part when its quantity is compared with that of other ingredients, and that there are no cells discernible made of any other material. The youngest cells, such as those in the first rootlets and leaves of germinating seed, contain the purest cellulose; with advancing growth and age this becomes covered by new homogeneous layers; other additions are heterogeneous, and varying in chemical composition, but one kind of matter is observed to be closely associated in some form or other with growing cellulose in all its varying forms and positions, namely a nitrogenised matter resembling the radicle which has been supposed to be the nucleus of the albuminous substances (protein). With advancing age and ceasing increase the cells assume a final rigid shape, which they maintain unaltered during the remainder of the existence of the organism of which they form part. The nitrogenised matter at

the same time leaves them almost entirely, and they become wood. Not rarely this wood, at the time of involution of the cell-life, receives an addition in the form of deposit or incrustation of mineral matter, mainly carbonate, and sometimes phosphate of calcium, silica, and potash and other salts. The chemical composition of cellulose finds its most simple expression in the formula  $C_6H_{10}O_5$ , but it is supposed that its molecule is a multiple of this formula, and therefore it is expedient to express its formula by  $n(C_6H_{10}O_5)$ . This formula also has the advantage of including the objects of those theories according to which there are several varieties of cellulose, some being represented as isomers, some as polymers of what might be termed the type.

In the growing parts of plants already developed cellulose is probably produced directly from and in the protoplasm, which, with its nucleus, serves as the centre of nutrition and multiplication, and as the mould, so to say, over which the actual cell of cellulose is formed. The cell is, in other words, the shell which protoplasm forms on its outside. But in seeds and tubers, and other organs effecting the propagation of the species by whatever means, a material for the formation of cellulose is ready for use deposited inside the cells, namely *starch*, or *amylon*. This material is not used by the very cells in which it is deposited, but is only stored there in a form insoluble for the time, but capable of being liquefied and carried to and employed in newly growing parts at the call of heat and light. Amylon also has the empirical formula of  $C_6H_{10}O_5$ , but most inquirers agree that this formula has at least to be doubled to express the considerations which arise from the simplest reaction. We are, however, justified in going further, and admitting, though hypothetically, that amylon, like cellulose, is best expressed by the formula  $n(C_6H_{10}O_5)$ , in which  $n$  is a factor not less perhaps than 5, and in given conditions probably more.

In considering amylon as a chemical individual it must be borne in mind that it is perhaps not identical with the whole of the product termed starch, but that a portion of the granules constituting this substance may be, as some maintain it is, actually cellulose, while another is amylon, and that in the concentric arrangement of the starch granules layers of these



substances alternate with each other. This discussion, however, is immaterial to our present purpose, because the fact remains, however the discussion may be decided, that the whole starch granule is available for fluidification by a ferment, and for transfer to parts newly formed or in course of growth.

In other parts of vegetables we find carbohydrates which are closely resembling cellulin and starch, and some of them are formed out of starch either by natural or artificial processes. These are the dextrins, gums, and sugars. Dextrin from wheat starch has, like this, the composition  $n(\text{C}_6\text{H}_{10}\text{O}_5)$ ; gum arabic or arabic acid, however, is at least  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ , while bassorin, the gum of tragacanth, remains, like dextrin,  $n(\text{C}_6\text{H}_{10}\text{O}_5)$ , and vegetable mucilage the same. In dextrin and gums the factor  $n$  may possibly be  $=1$  or  $=2$ , probably not more. The sugars have a great variety of formulæ, and are many in number. Pinite, or pine-sugar, is  $n(\text{C}_6\text{H}_{12}\text{O}_5)$ ; quercite, the sugar of acorns, has the same formula. Milk sugar, or lactose, is  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ ; cane-sugar has the same formula. Passing over a number of peculiar saccharoids occurring in rarer plants, we pause at the glucoses, the one which turns the polarised ray of light to the right being dextro-glucose,  $\text{C}_6\text{H}_{12}\text{O}_6$ , and the one which turns it to the left, or lævo-glucose, being perhaps of the same formula, possibly, however,  $\text{C}_{12}\text{H}_{24}\text{O}_{12}$ . Closely related to these is maltose,  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ , which will have to be considered more fully elsewhere. Next we notice mannitose, then mannite,  $\text{C}_6\text{H}_{14}\text{O}_6$ , an hydrogenised sugar, which can be produced by inserting hydrogen in a sugar with twelve atoms of hydrogen. Next follow some sugars which are common to plants and animals, namely inosite (phaseomannite) occurring in the muscles and brain of vertebrates, and dextro-glucose occurring in the blood and parts of animals; and scyllite in organs of the ray, shark, and prickly dogfish. Lastly we cast a glance at the glucosides, so termed because by chemolysis they yield glucose and other products. These are present in many plants under remarkable circumstances. Bodies like them are known to occur in animals, but are considered as rare and peculiar principles.

The carbohydrates of the order of gums and sugars do not as such serve for purposes of structure, but as vast as are the structure-functions of cellulose, so are the power-producing

functions of the sugars. The sugars are stores of matter or power for the accomplishment of the propagation of the species amongst plants. Roots and rhizomes, bulbs, stalks, leaves, and buds, but above all fruit abound in sugar. Lastly, in the living body of animals there is always sugar formed and present, whatever may be the mode of nutrition. Sugar seems at present incapable of being artificially metamorphosed backward into cellulín; neither does the metamorphosis appear to be effected in animals or plants.

The discovery by Schwann in 1840, that the elements of structure were the same in plants and animals, was followed in 1845 by the discovery of C. Schmidt (*Zur vergl. Physiologie der wirbellosten Thiere*, 1845, p. 62, *Ann. Pharm.* 54, 318; *J. Pr. Chem.* 38, 433) that some of the lower animals<sup>1</sup> were provided with parts consisting of cellulín. These were the tunicata (Lamark); in one species of these, and more particularly in the mantle of *Phallusia mamillaris*, cellulín, as it was believed to be, was met with. The discovery was extended by Löwig and Kölliker (*Ann. d. Sc. Nat.* 3 sér. v. pp. 193-232; *J. Pr. Chem.* 37, 439; *Compt. rend.* 22, 38, 1846), who, confirming Schmidt as to the presence of cellulín in *Phallusia mamillaris*, met with the same constituent in *Phallusia intestinalis*, *Phallusia monachus*, *Cynthia papillata*, *Clavelina lepadiformis*, *Diazona violaceum*, *Botryllus polycyclus*, *Pyrosoma giganteum*, and *Salpa maxima*, *Didemnum candidum*, *Aplidium gibbulosum*. They found the middle layer of the mantle of *Phallusia monachus* and *sulcata*, of *Clavelina lepadiformis* and *Aplidium gibbulosum*, to be composed of a glasslike transparent structureless substance in which many nuclei and crystals arranged in stars were imbedded; the external layer of the mantle they observed to be crowded with very large, elegant, round cells, with thin walls, enclosing a matter clear like water, but no nucleus. In *Clavelina lepadiformis* the entire mantle on the stalk as well as the sprouts is permeated by such roundish and oval cells without nucleus, to such a degree that the homo-

<sup>1</sup> Schmidt also believed to have found an infusorium, *Frustulia salina*, to contain cellulín. It was, however, afterwards shown that this creature is not an infusorium, but a plant.—Siebold, *Vergl. Anat. d. wirbellosten Thiere*, 1848, p. 238.

geneous substance, in which otherwise they are imbedded, has disappeared. Such layers of the mantle have then entirely the aspect of a vegetable tissue. In *Aplidium gibbulosum*, and *Botryllus violaceus*, the tender cells situated in the more external layer of the mantle contain calcic carbonate, which increases towards the periphery, so that the cells of the outermost layer appear completely calcified. In *Didemnum eandium* these calcified cells, which are here moreover provided externally with rays of calcic carbonate appear in such numbers that the entire stock of this compound ascidia appears to be penetrated by white starlike bodies. Similar to this appears, according to Milne Edwards (*Observ. sur les Ascidies composées*, p. 81, pl. 8, fig. 2, b), *Leptoclinum maenulosum*. In the mantle of *Diazona violaceum*, *Pyrosoma giganteum*, *Botryllus polyeyelus*, *Salpa maxima*, and *bicaudata*, these tender delicate cells are not seen, but the mainly homogeneous substance contains disseminated granules and nuclei, in *Pyrosoma* also cells with peculiar branches, and in *Diazona* coloured granules, and needle-shaped crystals or concretions of calcic carbonate. Such crystalline concretions in the shape of balls or stars are also present in *Salpa maxima*, and others in branched masses in *Salpa bicaudata*, but they are not soluble in hydrochloric acid, and are therefore probably not chalk but silicic acid. In *Botryllus* the homogeneous substance of the mantle is in certain places penetrated by peculiar serpent-like fibres, which twist in all directions, and on treatment with caustic potash are recognised to be wood fibres free from nitrogen. In *Cynthia papillata* the middle layer of the mantle consists of wavy wood-fibres, arranged longitudinally and peripherically, with interspersed nuclei, granules, crystals, and cells; the latter contain either brown pigment granules and a nucleus, or several endogenous cells, by which they recall the appearance of cartilage cells. The fibres of the middle layer of the mantle aid in the production of the thorny projections which appear on the otherwise horny-looking surface. In the mantle of *Cynthia pomaria* longitudinal fibres prevail, between which crystals, round pigment cells, and peculiar cells filled with yellow bodies are situated. With these cells are mixed a quite peculiar kind of cells, which are developed from pigment cells, and form thick

walls during their growth; these walls then break up into fibres, which latter now surround the cavity of the former cell in concentric layers. These fibrillated cell membranes react with potash as little as the principal substance; they are wood-fibre free from nitrogen; all other elements of the tissue disappear in the caustic alkali (Siebold, *Vergl. Anat.* p. 238, Anm. 4).

No such matter free from nitrogen and resembling cellulin was found by these observers in other molluscs, in annulates, helminths, echinoderms, discophores, polyps, and infusoria. In publishing their researches they expressed the apprehension that this discovery would be used by those who deny the existence of any precise demarcation between animals and plants, for the purpose of supporting the opinion that as regards chemical composition also there was no fixed difference between plants and animals. They therefore exerted themselves to prevent such a hasty conclusion, as it was termed, and pointed out particularly that the woody fibre of the tunicata was never contained in the mantle of these animals in a quite pure state, but was always yet mixed with animal elements, and that there existed no lower animal, which consisted in all its parts of cellulin (Siebold, *l. c.* p. 238, Anm. 1).

The researches of Löwig and Kölliker formed the subject of a report to the French Academy by Dumas, Edwards, Bous-singault, and Payen, published in the *Comp. rend.* 22, 581; *Ann. Sc. Nat.* 1846, 238.

Thus the matter rested for about twelve years, when Berthelot published some new researches on the peculiar substance of the mantle of *Cynthia papillata* (*Compt. rend.* 47, 227; *N. Ann. Chim. Phys.* 56, 149; *J. Pr. Chem.* 76, 371). As the result of these he maintained that this matter was not identical but only isomeric with cellulin. On chemolysis with sulphuric acid it yielded fermentable sugar and another matter not examined. He declared it to be rather analogous to chitin, termed it tunicin, and gave it the formula,  $C_6H_{10}O_5$ , or  $C_{12}H_{20}O_{10}$ .

The chemical details concerning tunicin will be summarised in a future article, where also will be discussed the supposed analogy to chitin, which Berthelot maintained on the basis of his own researches on that substance also.



For about the same time Berthelot found that chitin gave by chemolysis a fermentable sugar free from nitrogen. This was at first referred to the presence with chitin of some such body as tunicin, and Peligot (see references in the summary on chitin) actually endeavoured to find and extract the amyllum-like body which was supposed to yield this sugar. It is by no means decided whether such a body does not exist mixed with chitin. But chitin itself is nitrogenous, and therefore not at first sight so like cellulose as it would appear if it were free from nitrogen.

Schmidt, the discoverer of animal cellulin, had studied chitin also, and found that by thermolysis it gave out acetic acid and ammonia. But this observation was not appreciated by Städeler in his research on chitin; he occupied himself with constructing a new formula for this body on the basis of its empirical composition; he did not suspect that the sugar obtained by its chemolysis in a certain way, contained nitrogen. That under certain conditions it did contain nitrogen was only found in 1876. The chapter on chitin to be given below will explain how closely chitin is related to tunicin or animal cellulin, and to the amyloides of the vegetable world, which form the permanent structure of plants. It is indeed somewhat more complicated in chemical structure, inasmuch as it contains nitrogen; but even that is present in the rather simple form of amide; beyond this amide there are some simple radicles present which appear as fatty acids after chemolysis; but the radicle stock determining the nature of chitin is a body like cellulin, an amyloide. Though Löwig and Kölliker had not found any cellulin in helminths, a body was soon discovered in the peculiar cystic form of the taenia echinococcus, which resembled in these respects the cartilaginous covering of some of the lower animals, that by chemolysis with sulphuric acid it yielded dextro-glucose. The bladders of echinococci were found by Lücke (*Archiv Patholog.* 19, 189) to be made of a nitrogenous substance, which dissolved in hot water under pressure, like one of the permanent cartilaginous substances. The membranes of old bladders were in part insoluble in caustic alkali even on long boiling. The fully developed helminths contain probably no such matter in their cuticle, though they are, like the bladder,

covered with calcic carbonate. But they are covered, probably, with an amyloid, and at all events their parenchyma (in intestinal helminths) is pervaded by large quantities of so-called (hepatic) glycogen.

In the mollusca, provided with shells, a peculiar substance exists, mainly as the organic ingredient of the shells. This was for some time believed to be identical with chitin, but was at last separated by Frémy, and later by Schlossberger, and distinguished under the name of conchiolin. It is at once distinguished from chitin and hyalin by the amount of nitrogen, which is similar to that of the albuminous substances. The only crystal-

	Hyalin	Chitin	Byssin	Spongin	Conchiolin
C . .	44.55	45.69	—	47.44	50.0
H . .	6.64	6.42	—	6.30	5.9
N . .	4.81	7.00	13.5	16.15	17.5
O . .	—	—	—	—	26.6

lisable product as yet obtained from it is leucin; this would range it under the animal matter to be considered later on.

The byssus of the acephala was also supposed to contain chitin (Leuckart), but apart from its reactions, which are peculiar, it contains from 12.2 to 13.9 per cent. of nitrogen, and therefore differs from both chitin and conchiolin. The byssus of the mussel, when freed from impurity and a little calcic carbonate, is a mass of fibres shining like silk, and elastic like caoutchouc, and seems at first sight to have little physical similarity to other animal structures, except elastin.

Spongin has also yielded leucin and glycine, but not a trace of tyrosine. It has not yet been examined for an amyloid, but as some declare it to be identical with fibroin, which has been found to yield sugar, further examination may furnish information on this point. The nitrogen in spongin is 16.15 per cent. and this ranges the substance between byssin and conchiolin. Fibroin, the substance of silk, has yielded on chemolysis tyrosine (in the large quantity of 5 to 8 per cent.), leucin and other amide-acids, ammonia, sugar recognisable by an alkaline solution of cupric oxide, and especially, on long boiling, glycine. The composition of fibroin is similar to that of conchiolin,  $C_{15}H_{23}N_5O_6$



expressing its empirical formula. Heated with caustic potash it easily forms oxalic acid. It is well intermediate in chemical structure between the cellulins on the one, and the proteins on the other extremity of the scale.

Silk gelatin has yielded tyrosin, leucin, and serin,  $C_3H_7NO_3$  (amido-oxypropionic acid) which yields glyceric acid by treatment with nitrous acid. Silk gelatin is the outer covering of the silk fibroin, which forms the entire fibre; its composition is expressed by the empirical formula,  $C_{15}H_{25}N_5O_8$ ; it thus appears to contain a molecule of water and one of oxygen more than fibroin. The difference is, however, greater than that indicated by the one in hydrogen and oxygen; inasmuch as the radicle represented by serin has not yet been observed amongst the products of the chemolysis of fibroin. Mucin is the substance characterising the mucous secretions and excretions of a great number of animals. Snails secrete it in large quantities to prevent adhesion to objects in their progress; they lubricate their way with it; inversely almost all animals form it in their interior parts to prevent adhesion to them of foreign bodies, like food, or adhesion to each other in parts exposed to friction, or to the drying effects of the air. It exists in quantity in the glands forming the lubricating materials of the digestive canal, notably the salivary glands; but it is still more singular that the lubrication of tendons in their sheaths should be attained by it; and that the tough material of the tendons themselves, and of all connective tissue whatever, should contain a large amount of mucin. Its formula has not yet been attempted; it is separated from the albuminous matters by its insolubility in strong acetic acid. By chemolysis with mineral acids, or even by prolonged boiling with moderately strong acetic acid, it gives a kind of acid-albumin and grape-sugar, and probably other substances. The grape-sugar has been isolated and identified with alkaline copper solution, and with potash ley (Eichwald).

The analyses of mucin differ as yet too much from each other to allow the attempt of constructing a formula. Nitrogen is found by some at 8.5, by others at intermediate figures, 9.42, 10.01, 12.64, 13.22, 14.54, 17.69; not all of these mucins have been shown to form sugar. The mucin from the sub-maxillary gland dried and pulverised, and then mixed with very

dilute sulphuric acid, and heated over steam, gives a solution, even after 25 minutes' heating, and still more after 30 minutes, which, though a considerable amount of the mucin employed is yet undissolved, after removal of the sulphuric acid and concentration, reduces potassio-cupric tartrate and causes cuprous oxide to be deposited. When warmed with excess of soda ley alone the liquid turns brown; it likewise reduces bismuth oxide in alkaline solution as well as sulphindigotic acid. If, on the other hand, the heating of the mucin with dilute sulphuric acid be continued for a longer time, the quantity of the reducing substance which passes into solution decreases, and seems ultimately to disappear altogether. This reducing substance is not dissolved by ether, when its acid solution is agitated therewith; it is also insoluble in absolute alcohol, and is therefore different from grape-sugar and milk-sugar, from which it is likewise distinguished by its property of disappearing on prolonged heating with very dilute sulphuric acid (Obolenski, *Med. Chem. Unter.* 1871, 590.) But enough has been shown to make mucin a factor in the argument here proposed; it is an element of structure, and an agent of function, and from its qualities eminently fitted for both performances.

Mucin is also a product of diseased action, as it occurs in the fluid contents of colloid sacs, in sarcomatous, and in fibroid tumors.

Chondrinogen, or cartilage, is by itself insoluble in water. It constitutes the permanent cartilages, the bone cartilages before ossification, and the cornea of the eye. In growing stag's horn it is contained together with ossein, a mixture present in the bones of young individuals of all vertebrates. Rib-cartilage purified with dilute hydrochloric acid, when boiled with fuming hydrochloric acid, yields fermentable sugar (G. Fischer and Bödeker, *Ann. Pharm.* 117, 111; see also Schiff, *Ann. Pharm.* 119, 256). This sugar (chondrose) which Bödeker had formerly described as chondroitie acid (Schmidt's *Jahrbücher*, 90, 150), was found by De Bary to be lævorotatory and to differ from both dextro-glucose and lævo-glucose. Chondrin yields by fusion with potash oxalic acid, leucin, glycine, and an acid, which is fixed and gives a crystalline lime-salt. It sometimes forms a jelly with water, on cooling, like gelatin, therefore

like starch. The products of the chemolysis of chondrin under varying conditions are very imperfectly known, but nevertheless it is clearly marked as an amylonide.

Ossein, the organic basis of bones and teeth, is also in part the organic basis of reindeer's feet, stag's horn, and whalebone. In boiling water it is slowly converted into an equal weight of gelatin, more rapidly when prepared from the bones of young animals, and very speedily after addition of small quantities of acid. It contains—

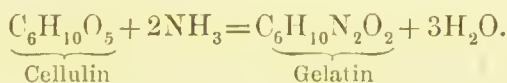
C 50·4; H 6·5; N 16·9; O 26·2.

The bones of fish and of aquatic birds contain besides ossein a substance insoluble in boiling water, yet apparently of the same composition as ossein. When these bones are freed from lime-salts by cold dilute hydrochloric acid, and the residue is washed free from acid, and then boiled with water, the ossein dissolves as gelatin, while a transparent elastic residue, having the form of the bones, remains undissolved (Frémy, *N. Ann. Chim. Phys.* 43, 51; *N. J. Pharm.* 27, 1).

Gelatin is also obtained from the gelatigenous tissues, the intercellular or so-called connective tissue, from tendons, ligaments, fasciæ, and lining membranes of sacs and cavities in the animal organism.

Elastic tissue and fibro-cartilage produce a somewhat peculiar glutin, and will be considered separately.

Already, thirty years ago, Hunt (*Amer. Journ. of Sc.*, Jan. 1848, p. 74, 109) perceived that when he added the elements of ammonia to those of cellulose or of amylose, and then deducted the elements of water, he obtained a product which had very nearly the formula of gelatin—



The percentic composition of a body  $n$  ( $C_6H_{10}N_2O_2$ ) would be—

C	.	.	50·70
H	.	.	7·04
N	.	.	19·71
O	.	.	22·55
			<hr/> 100·00

The calculated N is higher than the quantity found by analysis. Should gelatin be found to really contain some S, then this element would have to be substituted for some oxygen in the formula. Gerhardt, who is perhaps the only author who has noticed this speculation of Hunt (*Traité de Chim.* 4, 508) put it to a test, and made an interesting discovery. He caused isinglass to boil, during some days, with dilute sulphuric acid, and obtained ammoniac sulphate, and a considerable quantity of sugar (gelatose or ichthyocollose), which by fermentation was transformed into alcohol and carbonic acid (*comp.* Gerhardt, *Chim. organ. appliq. à la Physiologie végétale de M. Liebig*, p. 287.)

In the case of gelatin as in the cases of the albuminous substances the products of chemolysis, which are crystalloids, have been well studied; but those which are colloids, at least in the first instance, have been neglected. We shall study them closer hereafter; here it is important to remember that Scheele already, in attacking gelatin with nitric acid and heat, obtained oxalic acid, malic (saccharic) acid (questioned by Gerhardt, but without his giving grounds), a greasy and an astringent matter (Berzelius, *Lehrb. d. Chem.* 3rd edit. 9, 800.)

We shall see, in reviewing late researches on gluten, that it is proposed to distinguish and separate isinglass from ossein proper, and that therefore it is perhaps not safe to conclude from Gerhardt's experience that gluten from bones and other tissues of higher animals will also yield a fermentable sugar.

Gelatin (like pure cellulose), when purified by dialysis, is imputrescible in the ordinary sense, perhaps because, being free from salts, it affords no food to the organic putrescence ferments. It combines with colloid acids, and therefore Graham termed it a colloid base.

Keratin, or horn substance, the constituent of all kinds of coverings of the higher animals, exists in epidermis, epithelium, in hair, horns, nails, claws, hoofs, and feathers, mixed with a very great variety of colouring ingredients, as great perhaps as that of the colouring ingredients of the chitin of the articulates. The crystalloids obtained by its chemolysis which are best known are leucine, tyrosine, aspartic acid. The *aperçus* of similarity to amyloides in the bearing of keratin are numerous, but not yet sufficiently



precise to be used. But most remarkable is the bearing of the serpent's skin. This organ, when treated alternately with cold oil of vitriol and cold potash ley, whereby nitrogenous substances are dissolved, leaves a substance which is isomeric with cellulin, and yields fermentable sugar on boiling with dilute acids (De Luca, *Compt. rend.* 57, 437). Now this cellulin might be there as such, and not a product of decomposition of keratin, as is supposed by treating it in this place. It would then be analogous to the occurrence of cellulin in the skin (chitin) of the silkworm. For the same author found (*Compt. rend.* 53, 102) that the skin of the silkworm, and the brown coverings of the pupas which remain in the cocoons when the butterflies escape, are capable of yielding a substance isomeric with cellulin, which may be converted into glucose. When the caterpillars are boiled for several hours with strong hydrochloric acid, and this treatment is repeated three times with the residue, and the residue from these processes is washed with strong potash ley, then with water, and dried between 100° and 110°, a white light substance nearly free from nitrogen is obtained, which gradually diffuses in oil of vitriol, forming a colourless gummy liquid. This solution, added by small quantities to boiling water and boiled for an hour or two, yields fermentable sugar, which further reacts like glucose with common salt and potassio-cupric tartrate.

These forms of cellulin then might be admixtures of the keratin and chitin tissue respectively, and then they would show nothing as regards the chemical structure of the keratin. But even so the occurrence of cellulin in the skin of the serpent is a powerful argument in aid of the hypothesis of the amylo-nide structure of organised tissues. But it is of little consequence if we possess at present no direct evidence of the presence of an amylo-nide radicle in keratin, and in glutin from ossein, and other substances. For as they are derived from the higher albumin, and as albumin does indubitably contain an amylo-nide radicle, it is fair to assume that the derivatives also may contain it, unless indeed they have lost the amylo-nide radicle in the course of their being changed into structure-elements. But this is not likely, as their composition and bearing is too much like that of albumin.

We now come to the class of albuminous substances in which

the presence of an amylonide radicle can be proved *physiologically* by biolysis much better than by chemolysis. Of these albuminous bodies we have the white of egg, or albumin; the albumin of blood-serum, or serin; the albumin of flesh; the contractile matter of flesh, or syntonin, and the curdling matter or myosin, then paraglobulin and fibrin as natural educts from blood and other serous liquids; then casein from milk (with which some declare paraglobulin to be identical), then globulin from the crystalline lens, and globulin from hematoglobulin, or hemato-crystallin, and some abnormal products of disease not yet sufficiently studied.

All these by chemolysis yield products of which the greater number are crystalloids and well understood, while another part is not yet fully interpreted.

Bopp had found that when any of the albuminous substances is boiled with strong mineral acid, it yields a mixture of products containing tyrosin, leucin, leucinide, and some other crystalloids in smaller quantity. The non-crystallisable portion tastes sweet, does not undergo fermentation with yeast, evolves hydrothion when boiled with potash, and afterwards acidified with acetic acid, and forms a violet solution with potash and cupric sulphate or potassio-cupric tartrate.

These inquiries were subsequently extended, and compound volatile alkalies, fixed alkaloids, such as fluorescentin, and fluopittin, a substance recalling the bearing of biliary matters by its spectrum, and others in smaller quantity were discovered.

In these chemolyses the process did probably go beyond the stage at which the amylonides are, or one of them is, separated, but not destroyed. For Schützenberger (*Chem. Centr.bl.* 1875, p. 614 *et seq.*) decomposed albumin by boiling it for one hour only with very dilute sulphuric acid, and found amongst the soluble products a nitrogenous body, which strongly reduced Fehling's fluid, was precipitated by ammoniacal lead acetate, gave no precipitate with mercuric nitrate, and seemed to him to be glucose or some such body. He further decomposed albumin with barytic hydrate (*l. c.* p. 631), under pressure at a temperature of from 150° to 200°, and obtained by various manipulations, to be described elsewhere, a considerable quantity of matter, 3 to 4 grms. from 70 grms. dry coagulated albumin,



which had a clearly sugar-like taste, did not evolve ammonia when heated with potash, was soluble in water, insoluble in absolute alcohol; when heated on platinum foil, it swelled up, and charred, without giving any sublimate, and smelt like charred bread. This product, fully exhausted with alcohol, contained

				Calculated for theory below.
<i>a.</i>	C . .	44.2 per cent.		44.7 per cent.
	H . .	7.6 „		7.7 „
	N . .	14.8 „		15.0 „

Another similar product obtained in the same manner from hemialbumin contained

<i>b.</i>	C . .	42.4 per cent.
	H . .	8.0 „
	N . .	14.5 „

From this statement it appears that one such reducing body was obtained from albumin, by the side of hemialbumin, under the influence of sulphuric acid, another from hemialbumin itself by secondary chemolysis with baryta; therefore two such nitrogenous copper-reducing nuclei seem to be present in albumin.

Schützenberger considers the body *a* to be a compound of  $C_{12}H_{28}N_8O_4$  with  $3C_4H_8NO_2$  (glykoprotein *a*)— $H_2O$ . The compound  $C_{12}H_{28}N_8O_4$  or  $C_6H_{14}N_2O_4$  he explains as an amide of cellulose; and he thinks that from this body might be derived the small amount of dextrin which was found in a third experiment, chemolysis of hemiprotein by baryta. In this experiment a mercuric nitrate precipitate yielded on decomposition matters from which absolute alcohol extracted a sugar-like amorphous body, which contained

	Exp. III. <i>a.</i>				Exp. III. <i>b.</i>	
	1	2	3	4	5	6
C . .	49.51	48.79	—	—	52.8	—
H . .	6.78	6.86	—	—	6.83	—
N . .	—	—	13.45	12.8	—	14.23

Besides this nitrogenised body the baryta-salt (in experiments like Exp. III.) always contains a certain quantity of a non-

nitrogenised body which is insoluble in alcohol, is precipitated by ammoniacal lead acetate, and by boiling with dilute sulphuric acid is metamorphosed into a body which reduces Fehling's fluid; the original body does not reduce Fehling's fluid, but gives oxalic acid on treatment with nitric acid. The body gave on analysis

C	.	.	42.8 per cent.
H	.	.	6.56 „

These figures are near those which would be yielded by dextrin. Schützenberger surmised that the occurrence of this cellulin-like body in the baryta chemolysis stood in connection with the occurrence of a body reducing Fehling's fluid which was found in the sulphuric acid chemolysis. But although he thus recognised that 'the molecule of albumin contains a small quantity of a cellulin amide,' he neglected this ingredient in all his following calculations and theories, and came to the result that albumin was a complex ureide; the further details of structure he explained upon this basis, and in consequence did not arrive at the explanation of the structure of albumin as we shall have to formulate it.

The amyloid nature of the albuminous substances is proved by the so-called glycogenetic function of the liver, and the lactopoetic function of the mammary gland of the carnivora. It is further proved by the growth and development from eggs to the adult state of maggots reared on boiled white of egg exclusively. These remarkable proofs science owes mainly to Bernard. But he did not draw the conclusion which is here propounded, and, like other inquirers in his time, considered the formation of sugar from albumin an accidental vicarious act, performed when no carbohydrates were at hand to suit the wants of the body.

The paralbumin of Scherer, obtained from ovarian cysts, contains, besides albumin, a body resembling liver-starch, in this that it dissolves in water to a turbid fluid, is precipitated by alcohol, coloured brown by alkalies and yellow by iodine, and when boiled with dilute sulphuric acid yields a product which reduces cupric and bismuthic oxide. At the same time there is an albuminous body present which can be precipitated by

neutralisation and boiling ; and then the liquid contains another probably albuminous body, which on boiling with mineral acids yields a flocculent brown precipitate and a solution reducing, after neutralisation, copper, bismuth, and indigo. The original substance gave by analysis 49·7 per cent. C, 7·6 H, and 7·4 to 8·8 N. This body is therefore an amyloid, similar to the lower forms of mucin ; but as a pathological product it deserves special study.

The following list of organoplastic substances is so arranged that it begins with the simplest, namely, cellulin, and ends with the most complicated, i.e. albumin. As they are considered here principally in their hypothetical character of substances formed upon the type of amyloid, they are termed amyloidides, and the sugars to which they give rise, or are supposed to give rise, by chemical or physiological agencies are named in connection with them on the right of each term.

*Organoplastic animal amyloidides and sugars to which they give rise.*

Cellulin . . . . .	Dextrose.
Glycogen or hepatic amyloid	Dextrose.
Tunicin . . . . .	Tunicose.
Chitin . . . . .	Chitose.
Conchiolin . . . . .	Conchiolose.
Hyalin . . . . .	Hyalose.
Fibroin . . . . .	Fibrose.
Serin . . . . .	Serinose.
Spongin . . . . .	Spongose.
Keratin . . . . .	Keratose.
Chondrin . . . . .	Chondrose.
Cerebrin } . . . . .	Cerebrose.
Phrenosin }	
Kerasin }	
Gelatin . . . . .	Gelatose.
Ichthyocollin . . . . .	Ichthyocollose.
Mucin . . . . .	Mucinoses.
Paralbumin . . . . .	Paralbuminose.
Albumin . . . . .	Albuminose (lactin), or albumino-dextrose.

These names seem convenient as designating the origin of each variety of sugar; they or any of them will have to be changed as soon as it can be shown that the sugar corresponds to a known variety of more general occurrence. Some terms may be doubtful, e.g. the reducing body, supposed to have been a sugar, from serin, or silk gelatin, may have been sericin, which is not a true saccharoid but a hemi-saccharoid or glyceronide, and thus resembles the fats and phosphorised substances of the brain and other tissues. Again in the case of the sugar obtained from gelatin it might be possible that one of the reducing amido-acids (glutamic and asparaginic) had simulated sugar, a doubt which requires further attention. This doubt could, however, not apply to the sugar obtained from isinglass, which did not only reduce but by fermentation gave alcohol.

Hepatic amylon or glycogen occurs twice in the list, namely, as an organoplastic substance by itself and giving rise to dextrose, and as a product of the decomposition of albumin, as albuminose, or albuminous dextrose. Even if these bodies should be identical, which is not very likely, the repetition will be a concession to caution, as they might be formed in different places by different processes (lactin, in the mammary gland, glycogen in the liver).

Some of the sugars differ greatly from each other at first sight. Supposing the properties of dextrose to be well known, we can compare the other bodies to it. The sugar from chondrin, chondrose, turns the plane of polarised light to the left; lactin turns to the right, but is a disaccharonide, one molecule of it yielding by hydration two molecules of lactose. Cerebrose is probably isomeric with dextrose, reduces copper solution, turns the ray of polarised light to the right, and crystallises from water in crystals which do not lose water at  $100^{\circ}$ , while dextrose crystals at that temperature give up a molecule of water. All these differences and their causes will be discussed or investigated in detail hereafter.

The chemical constitution of the fats and of the phosphorised substances is determined by a radicle which by chemolysis to ultimate crystalloids appears as glycerin. This radicle receives additional importance in relation to our present argument, by the fact that it can be considered as a half-sugar, so to say, a

hemisaccharon; its formula is about half that of sugar, its function is that of a tridynamic alcohol, while the functionally most developed sugar is a hexadynamic alcohol. Moreover, two molecules of glycerin (so it has been stated) may combine and form sugar. The substitution of three molecules of fatty acid for three molecules of hydroxyl in glycerin produces the vegetable and animal fats. In the phosphorised substances, however, only two molecules of hydroxyl are substituted by fatty acids; the third hydroxyl is substituted by phosphoric acid, and to this is attached a collateral chain of smallest radicles, the complex of which is termed neurin or cholin. Fats and phosphorised substances may, therefore, be classified as hemisaccharonides, or glyceronides, or as ethers of the alcohol glycerin with simple or compound acids. To this class of bodies serin or silk gelatin may possibly belong: it contains and yields by chemolysis a glyceritic amide (sericin) serosamide, which is analogous to chitosamide thus far, that we might signalise it by the term hemichitosamide, were not the amide wanting in the ideal second half of the ideally severed saccharoid radicle.



## II.

ULTIMATE CRYSTALLOID PRODUCTS OF THE CHEMOLYSIS OF THE ORGANOPLASTIC SUBSTANCES, PARTICULARLY BY CAUSTIC BARYTA. (*Summary and Additions.*)

1. *Introduction. Researches of O. Nasse. Researches of P. Schützenberger. Description of his process.*—The forcible decomposition of albuminous substances by caustic baryta with a view of obtaining information concerning their constitution and differences was initiated by O. Nasse (*Arch. Physiol.* vols. vi. vii. viii.) and led to the information that the organoplastic substances under the influence of this reagent at the ordinary pressure of the air and a temperature of 100° lose a definite portion of their nitrogen in the form of ammonia, and that the quantities thus evolved vary with different albuminous substances. Nasse obtained the following relations between the nitrogen evolved and that not evolved, expressed by the amount of the former divided by the latter:—

For casein	.	.	.	.	0.033
„ serum albumin	.	.	.	.	0.0889
„ gluten	.	.	.	.	0.275

Nasse treated the albuminous substances first with fuming nitric acid, and then boiled them with excess of baryta during 2½ hours. This did not enable him to obtain definite products either intermediate or final, of the cleavage of the substances operated upon. When Schützenberger operated in this way, and continued the boiling for 120 hours, he obtained from 100 grms. dry coagulated albumin 1.4 to 2 grms. nitrogen, in the form of ammonia. The 100 grms. albumin contain, however, 15.8 grms. nitrogen; there remained, therefore, from 14.4 to



13.8 grms. nitrogen in the matters mixed with the baryta. The proportion  $\frac{1.4}{14.4} = 0.097$  approaches the proportion found

by Nasse, but is somewhat higher; the proportion  $\frac{2}{13.8} = 0.145$

is, however, far removed from it. From this it follows that the results obtainable by boiling an albuminous substance with caustic baryta are not of great importance. They point, however, unmistakably to a great difference in the constitution of the bodies above enumerated.

Schützenberger (*Bull. Soc. Chim. Paris* [N.S.] 23, 161; and *Ann. Chim. Phys.* 16, 1879, 289) attacks the albuminous bodies by baryta in a more forcible manner. He mixes one part of coagulated substance (corresponding in most of his experiments to 100 grms. of dry substance) with from three to six parts (therefore corresponding to 300–600 grms.) of crystallised baryta hydrate, and three to eight parts (*i.e.* 300 to 800 c.c.) of water, encloses the whole in a cylinder of cast steel lined with silver, and heats it during periods varying from six hours to six days, to a temperature of from 160° to 250°. The most complete chemolysis is obtained with the largest quantities of baryta at the highest temperature in the shortest time.

The perfectly cool contents of the cylinder are distilled until all *ammonia* has been expelled and condensed in two successive receivers, the last one containing excess of hydrochloric acid. On the top of the alkaline liquid in the first condenser floats a small quantity of a colourless oil (*albuminol*) containing, as will be shown more in detail lower down, *pyrrol*, an *oxygenated body*, free from nitrogen, and a *sulphur compound*. This oil is separated mechanically from the ammonia before the latter is mixed with the acid contents of the second receiver. For the albuminol is soluble in dilute hydrochloric acid, and quickly changed by it, reddish brown flocks of pyrrol red being deposited. The contents of the two receivers are mixed, care being taken that the mixture be acid. One-tenth of the hydrochloric acid solution, corresponding to ten grms. of albuminous substance, is used for a quantation of the ammonia as platino-chloride. Of this, on an average, under the foregoing conditions six grms. are obtained,

The barytic mixture, from which all oil and volatile alkali has been expelled, is now filtered through a weighed filter, and the insoluble precipitate, consisting of baryum *carbonate*, *oxalate*, *sulphite*, is washed with hot water and weighed. It may contain some baryum soap in case the albuminous substance had not been completely freed from fat. It may therefore be purified by solution in hot hydrochloric acid, filtered to remove some brown flakes and any fatty acids, and then be made alkaline with caustic ammonia. On standing in a place free from carbonic acid during twelve hours the solution deposits crystallised oxalate of baryum, which is dried and weighed. But even this purified deposit may contain some phosphates of earths, from which the oxalate can be separated by boiling the precipitate (crude or purified) with sodic carbonate, filtering, acidifying the solution with acetic acid, and precipitating with calcic chloride, and treating the precipitate further in the usual manner for the quantation of oxalic acid.

The clear alkaline barytic filtrate from the above-named baryum salts is now completely precipitated by a current of carbonic acid gas, filtered, heated to boiling to destroy a little baryta bicarbonate, filtered again, and accurately precipitated with dilute sulphuric acid, so that it contains neither baryum nor sulphuric acid. The barytic sulphate is collected on a filter, washed and weighed. The filtrate, which contains no mineral substances, is distilled in a vacuum to dryness. The distillate is neutralised, and, after concentration, distilled a second time, and in this distillate the *acetic acid* is estimated by neutralisation with normal soda solution. The solid residue which contains *all fixed organic products* of the cleavage of the albuminous substance, is a mixture of amidated compounds, which for the sake of brevity is termed *the amido-mixture*. It is completely dried in a current of air at 100° and weighed.

Before proceeding to the consideration of the analytical operations to which this amido-mixture is subjected, it is requisite to record, that the baryta carbonate, obtained by passing carbonic anhydride into the solution filtered from the carbonate, oxalate, &c., after the volatile alkalies and oils have been distilled off, retains with pertinacity some quantity, amounting to

from 5 to 8 per cent. of the amido-mixture, all washing with boiling water notwithstanding. This necessitates that the baryum carbonate should be decomposed with exactly its equivalent of sulphuric acid, that the sulphate formed should be edulcorated, that the filtrates, free from baryum as well as sulphuric acid, should be evaporated in vacuo, and that the dry residue thus obtained should be added to that of the amido-mixture.

The whole of the amido-mixture thus obtained is intimately mixed by powdering, and subjected to the following analytical operations:

- a. The C, H, and N are estimated by elementary analysis.
- b. The proximate constituents are isolated as far as possible, and subjected to elementary analysis.
- c. The quantity of tyrosin and, as far as possible, that of leucin (these bodies being the least soluble and most crystallisable ones) is estimated.

In this manner a series of data are obtained, which may serve to explain the phenomena of the chemolysis, and aid in the attempt to ascertain the constitution of the organoplastic substances. These data are discussed in the following, under the relative headings.

2. *Nitrogen separated in the form of Ammonia from different organoplastic substances.*

a. Coagulated albumin, produced by dissolving mercantile scale albumin in water, adding acetic acid, and precipitating by heating on the steam-bath. N=maximum 4.41 per cent.; minimum 3.46 per cent.; mean 3.93 per cent.				
b. Serum albumin	.	.	„	3.96 „
c. Casein .	.	.	„	3.54 „
d. Fibrin from horse's blood	.	.	„	4.83 „
e. Muscle fibrin, veal	.	.	„	4.30 „
f. Gluten exhausted by boiling alcohol	.	.	„	4.44 „
g. Hemiprotein	.	.	„	3.60 „
h. Ossein .	.	.	„	3.01 „
i. Gelatin	.	.	„	2.55 „

From the foregoing it is evident that the amount of nitrogen ejected from different albuminous substances varies

within certain limits. These limits and the conditions of the variations are not yet determined. It is, however, already certain that the maximum of nitrogen is obtained only by the agency of much baryta, at very high temperatures; and that the nitrogen evolved rises with the quantity of reagent, the time of its action, and the temperature at which it acts, in a certain ratio, which is almost the same ratio as that in which the quantity of carbonic acid evolved at the same time rises, but is different from the ratio in which oxalic and acetic acid rise.

3. *Weight of the mixed baryum precipitate, and separate weights of the baryum carbonate and baryum oxalate.*—The precipitate also contains sulphurous acid, which has not been estimated; further, possibly fatty acid and phosphates of earths under the conditions already alluded to above; further, it may contain some products of the decomposition of glass, if the boiling has been effected in glass vessels. From this composite nature of the precipitate, it follows that no very safe conclusions can be drawn from the comparison of its weight with that of the evolved ammonia. Coagulated albumin gave in eight experiments (200°) figures which varied between 28 and 30 grms. Fibrin from horse's blood gave 32 grms., albumin from horse's blood 30 grms., gluten exhausted with boiling alcohol 25 grms.

The composition of the baryta precipitate obtained at 160° to 200° is constant for one and the same specimen of organoplastic matter, but changes with the nature of the substance.

a. *Coagulated Albumin.*—Some sorts of mercantile white of egg gave only traces of oxalate; another white of egg, with which five experiments were made, gave—

Nitrogen mean . . . . .	=	3.84
Total bar. precipitate, mean	=	30.4

The mixed baryum precipitate contained for 30 grms.—

Barytic carbonate . . . . .	20	grms.
„ oxalate . . . . .	5.7	„
Total . . . . .	25.7	„

A third variety of albumin yielded—



Nitrogen . . . . .	3.9 grms.
Crude bar. precipitate . . . . .	<u>30.0</u> „
Containing—	
Barytic carbonate . . . . .	14.5 „
„ oxalate . . . . .	<u>12.5</u> „
Total . . . . .	27.0 „

It is therefore clear that the relation between the nitrogen evolved and the crude precipitate is nearly constant, but that the latter may contain very different proportions of oxalate and carbonate. These phenomena can be explained if it is

assumed that the oxamide group  $\begin{matrix} \text{NH}_2 \\ \text{NH}_2 \end{matrix} \rangle \text{C}_2\text{O}_2$  can replace partially the urea group  $\begin{matrix} \text{NH}_2 \\ \text{NH}_2 \end{matrix} \rangle \text{CO}$  just as in felspar minerals the alkaline metals can replace each other in very different proportions. Or the existence of two kinds of albumin may be assumed, the one deriving from urea, the other from oxamide, which are mixed in ordinary white of egg in different proportions. The proportion between the evolved nitrogen and the total weight of the carbonate and oxalate approaches very closely that which should be obtained if it is assumed that the evolved nitrogen is derived from the decomposition of the urea or of the oxamide.

Thus the experiments given above, concerning the second specimen of albumin, gave—

Mean weight of nitrogen . . . . .	3.8
„ baryum carbonate . . . . .	20.0
„ „ oxalate . . . . .	5.7
Nitrogen calculated from carbonate . . . . .	2.84
„ oxalate . . . . .	<u>0.71</u>
Total of calculated nitrogen . . . . .	3.55

a figure which differs from the quantity found (3.8) only by 0.25.

The third variety of albumin gave—

Nitrogen calculated from the carbonate . . . . .	2.05
„ „ „ oxalate . . . . .	<u>1.55</u>
Total of nitrogen calculated . . . . .	3.60

a figure which differs from the quantity found, 3.90, only by 0.3.

*b. Casein.* Casein easily retains a little fat, which appears as baryum soap in the baryum precipitate. Oxalate and carbonate have therefore to be estimated after purification. 100 grms. of casein, dry, gave—

Baryum oxalate	.	.	.	17.5	grms.
„ carbonate	.	.	.	7.6	„
				<u>25.1</u>	„
Nitrogen calculated from oxalate	.			2.17	grms.
„ „ carbonate	.			1.08	„
				<u>3.25</u>	„
Total nitrogen calculated	.			3.25	„
Nitrogen found	.	.		3.54	„

These figures differ by only 0.3.

*c. Serum albumin from horse's blood.*

Crude precipitate 30 grms., containing—

Baryum oxalate	.	.	.	16.5	grms.
„ carbonate	.	.	.	10.5	„
				<u>27.0</u>	„
Nitrogen calculated from oxalate	.			2.05	grms.
„ „ carbonate	.			1.50	„
				<u>3.55</u>	„
Total nitrogen calculated	.			3.55	„
Nitrogen found	.	.		3.95	„

Difference 0.4.

*d. Fibrin from horse's blood.*

Crude precipitate 30 grms., containing—

Baryum oxalate	.	.	.	11.5	grms.
„ carbonate	.	.	.	20.5	„
				<u>32.0</u>	„
Nitrogen calculated from oxalate	.			1.43	„
„ „ carbonate	.			2.91	„
				<u>4.34</u>	„
Total nitrogen calculated	.			4.34	„
Nitrogen found	.	.		4.80	„

Difference 0.46.



*e. Hemiprotein.*

Crude precipitate 30 grms., containing—

Baryum oxalate	.	.	.	14.7	grms.
„ carbonate	.	.	.	11.5	„
				<u>26.2</u>	„
Nitrogen calculated from oxalate	.			1.82	„
„ „ carbonate				<u>1.63</u>	„
Total nitrogen calculated	.			3.45	„
Nitrogen found	.	.	.	3.60	„

Difference 0.15.

*f. Vegetable fibrin* (gluten exhausted with boiling alcohol).

Crude precipitate 25 grms., containing—

Baryum oxalate	.	.	.	8.0	grms.
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The precipitate showed an unexpected peculiarity in this that it evolved only little carbonic acid on treatment with hydrochloric, but set free a considerable amount of a liquid fatty acid. It is necessary to make further experiments to see whether the fatty acid found originates with the gluten itself or with a fatty body present as an impurity only.

*g. Ossein* (bone-dust from the manufacture of buttons, exhausted with dilute hydrochloric acid).

Crude precipitate from 100 parts dry, 26 grms. containing—

Baryum oxalate	.	.	.	5.0	grms.
„ carbonate	.	.	.	15.0	„
				<u>20.0</u>	„

There was a baryum soap present, which explains the difference of 6. grms.

Nitrogen calculated from oxalate	.			0.62	grms.
„ „ carbonate				<u>2.13</u>	„
				2.75	„
Nitrogen found	.	.	.	3.01	„

Difference 0.36.

From these experiments the conclusion seems admissible that generally the nitrogen evolved in the form of ammonia and estimated directly exceeds by 0·3 to 0·4 per cent. of the organoplastic substance, or by one-tenth of its own quantity, the amount which is calculated from the oxalate and carbonate. But at higher temperatures, *i.e.* 250°, and with an increased amount of baryta (say 100 grms. albumin with 600 grms. baryta, 400 grms. water, heated to 250° during six hours) the quantity of nitrogen in ammonia rises to 4·41 per cent., the baryta carbonate to 12·5 per cent., and the oxalate to 24·2 per cent. These conditions, as regards albumin, will be stated below in detail under the section 5 relating to acetic acid.

4. *Weight of the baryum which is not precipitable by carbonic acid, corresponding to the strong acids contained in the amido-mixture.* This datum is one of great constancy for the different organoplastic substances. There was found baryum sulphate—

For 100 grms. egg albumin .	. 22·5–24·0
„ „ serum albumin .	. 25
„ „ casein .	. 24
„ „ fibrin .	. 24
„ „ hemiprotein .	. 24
„ „ muscle fibrin .	. 22·2
„ „ gluten .	. 30
„ „ ossein .	. 13·2

Gluten is therefore distinguished by an excess of 6 grms., and ossein by its giving only about half the amount of sulphate which the albuminous substances yield in this reaction.

5. *Acetic acid evolved.*—With regard to this datum the different albuminous substances show peculiarities. The quantities of the acetic acid estimated directly by neutralisation of the distillate with normal soda solution (containing one equivalent of sodic hydrate [ $\text{NaHO}=40$ ] in the litre) were the following :—

				Parts from 100
Albumin, coagulated	(150° and 3 parts of baryta)			3·4
„	„	(250° and 6	„	) 4·2
„	„	(250° and 6	„	) 5·1
Casein .	.	(150° and 3	„	) 3·36
Blood fibrin .	„	„	„	3·6
Serum albumin	„	„	„	3·48
Vegetal fibrin .	„	„	„	2·00
Ossein .	.	„	„	1·44
Ichthyocollin .	„	„	„	1·3
Chondrin .	„	„	„	4·7
Fibroin .	„	„	„	2·0
Goats' hair .	„	„	„	2·1
Human hair .	„	„	„	4·2
Wool .	.	„	„	3·12

The quantity of oxalic and acetic acid (containing a trace of formic) evolved from albumin (and probably from the other substances) rises with the amount of baryta and the temperature employed.

100 grms. albumin chemolysed under the following conditions gave—

Baryta	Water	Temperature	Time	N in NH <sub>3</sub>	Ba CO <sub>3</sub>	Oxalate	Acetic acid
grms.	grms.	Centigrade	hrs.				
200	250	140–150°	12	3·1	10·5	5·0	2·7
300	250	175–180°	48	3·95–4·03	10·8	8·0	3·48
300	250	200°	120	3·95	11·0	8·6	3·78
200	250	160°	28	4·1	10·8	8·6	3·66
500	300	{ 175°	12	4·36	?	17·5	4·20
		{ 260°	2				
500	400	175°	48	4·03	10·7	17·6	4·92
500	400	175–180°	120	4·02	10·9	17·3	4·65
600	400	250°	6	4·41	12·5	24·2	5·4

6. *Elementary analysis of the amido-mixture, being the albuminous substance minus carbonic, oxalic, and acetic acid, ammonia, sulphur, plus water.*—The product obtained by evaporating the solution in vacuo was always feebly coloured, friable, hygroscopic, and free from mineral substances.

a. Coagulated albumin :—

C=48·69

H= 7·95 to 7·82

N=11·8 to 11·9

*b.* Coagulated serum albumin :—

$$C = 48.83$$

$$H = 7.76$$

*c.* Casein from cow's milk :—

$$C = 49.84$$

$$H = 7.89$$

*d.* Hemiprotein :—

$$C = 49.1$$

$$H = 8.11$$

*e.* Fibrin from horse's blood :—

$$C = 48.9 \text{ to } 48.99$$

$$H = 7.90 \text{ to } 7.84$$

$$N = 12.1$$

*f.* Gluten (vegetal fibrin) :—

$$C = 47.4$$

$$H = 7.6$$

*g.* Muscle fibrin from veal :—

$$C = 45.6$$

$$H = 7.86$$

*h.* Ossein :—

$$C = 43.7 \text{ to } 44.3$$

$$H = 7.42 \text{ to } 7.67$$

$$N = 13.2$$

The results are nearly identical as regards egg albumin, serum albumin, casein, blood fibrin, and hemiprotein. The small differences arise from a greater, or lesser proportion of tyrosin in the amido-mixture. In fact, casein yields more tyrosin than albumin, and blood fibrin yields an amount intermediate between the two. The amido-mixture of ossein possesses a different composition. The muscle fibrin of veal was more a mixture of different tissues than a unitary albuminous matter, and contained an uncertain amount of gelatigenous tissue, by which the low amount of carbon is explained.

The numbers which have been found for the amido-residue from albuminous bodies proper (albumin, casein, &c.), can be expressed by the following synopsis :—

		Found	Theory
C	. .	48.4	48.35
H	. .	8.0	8.00
N	. .	12.5	13.50
O	. .	31.1	30.15

leading to the formula  $C_{27} H_{54} N_6 O_{13}$  or to a multiple of that formula.

7. *Quantation of Tyrosin and Leucin.*—The quantation of tyrosin may be made by treating the dry amido-mixture with a mixture of 4 parts of water and one part of alcohol in the cold. The insoluble residue is dissolved in ammoniacal water, and the ammonia expelled by boiling, whereupon on cooling tyrosin crystallises almost entirely in long white needles, which are collected on a filter, dried, and weighed. In this manner 100 parts of the following bodies yielded tyrosin

. Albumin . . .	2.03 to 2.4 parts
Casein . . .	4.12
Hemiprotein . . .	2.2
Fibrin from horse's blood . .	3.2 to 3.5
Vegetal fibrin . . .	2.0

Another mode of extracting tyrosin and leucin from the amido-mixture is the following. The solution, after removal of all baryta precipitable by carbonic anhydride, and before the application to it of any sulphuric acid, is evaporated until a crystalline pellicle forms on its surface, and allowed to cool. The crystals which form are a mixture of tyrosin and leucin, amounting to from 25 to 30 per cent. of the albuminous substance employed. They are separated by the ordinary well-known methods.

Schützenberger at first thought that as tyrosin is present in the molecule of albumin, &c., in only small proportion, it might be left out of the calculation when the general character of the reaction is considered. In this, however, those would not agree who also take the physiological and pathological significance of tyrosin as a cleavage product of albumin into account, and remember that in the healthy body tyrosin is further destroyed, and yields probably aromatic products of peculiar significance as regards the composition of the excretions. We shall see at the conclusion



of this article how Schützenberger has lately adapted his theory to this requirement. The weight of leucin is obtained approximately, though less accurately than that of tyrosin, by exhausting the dry amido-mixture with hot alcohol of 90 per cent. strength, and collecting the crystals which form on cooling of the sufficiently concentrated liquid. The same quantity is obtained when the crystals are weighed which separate when a sufficiently concentrated solution of amido-mixture is allowed to cool. In this manner there are found for albumin 24 to 26 grms. of a mixture of leucin and leucein.

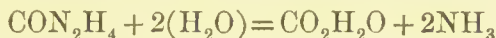
8. *Further consideration of the amido-mixture and its ingredients.*—In view of the almost complete identity of composition of the amido-mixture from the principal albuminous substances, and leaving out of consideration vegetal fibrin, which shows certain peculiarities to be further inquired into, it may be assumed as highly probable that these mixtures are qualitatively as well as quantitatively identical. This leads to the assumption that the albuminous substances have a common nucleus, which has the same constitution in all. The differences between the albuminous substances would in that case depend upon the nature and quantity of the secondary substances which are combined with these nuclei.

This view has the value of a good working hypothesis, and effects a revival of the protein theory of Mulder, though with the difference that the constitution of the common nucleus is accounted for. More or less complete substitution of the urea group by the oxamide group, varying quantities of tyrosin, the intervention of certain peculiar secondary groups, such as that of glutamic acid, the occurrence of a fatty acid in gluten, are sufficient to account for at least some of the differences of the albuminous substances.

Ossein and gelatin belong to a different type of compounds, the nuclei of which, though they may have some analogy to those of the albumins proper, are yet different. All the fundamental data of these bodies differ from those of the albuminous substances. The analysis of the amido-mixture shows, moreover, that lower members of the homologous series  $C_nH_{2n+1}NO_2$  enter into the molecule.

The quantity of water which combines with an albuminous

substance previous to its cleavage is determined by the number of nitrogen atoms contained in the molecule. If the decomposition of urea is expressed by the equation



then it is permitted to say that for every atom of nitrogen contained in the albumin a molecule of water enters at some time of the entire reaction. The amido compounds, therefore, which form exclusively the mixture which has remained in solution, exist in albumin and its congeners as imides.

The amido-acids contained in the amido-mixture belong to three series :

1.  $\text{C}_n\text{H}_{2n+1}\text{NO}_2$ , series of leucin.
2.  $\text{C}_n\text{H}_{2n-1}\text{NO}_2$ , series of the amido-acids of the acrylic series.
3.  $\text{C}_n\text{H}_{2n-1}\text{NO}_4$ , series of asparaginic acid.

The first series may for the purpose of brevity be termed that of the leucins, the second one that of the leuceins.

In the albuminous substances the leucins and leuceins are present in such relative quantities that the nitrogen of their sum is equally distributed between both classes of bodies ; therefore the general formula of the amido-mixture can, leaving out of sight the bodies of the asparaginic acid series, be expressed by  $\text{C}_n\text{H}_{2n}\text{NO}_2$ . The leucins and leuceins form the principal bulk of the amido-mixture (obtained by baryta chemolysis ; by hydrochloric acid chemolysis a much greater proportion of the members of the asparaginic acid series is obtained). The amount of the matters belonging to the asparaginic acid series can be approximately estimated by a quantation of the oxygen which is present in excess of the proportion  $\text{N} : \text{O}_2$ . We do not propose here to give the details of the operations required to separate the various bodies or groups of bodies from each other, as they are most complicated, and yet not very effective in all their parts, as shown by the uncertainty concerning the individuality of leucein, to be referred to more at length below under tyro-leucin. Fractional crystallisation from water and alcohol is the principal means employed to separate the leucins and leuceins. Amido-acids which are close to each other in a series, can be distinguished only by elementary analysis. Microscopic exa-

mination and proportions of solubility also give some slight assistance. By various processes there were isolated the following bodies; or when they were not isolated their presence in mixtures was shown by the results of elementary analyses.

Tyrosin,  $C_9H_{11}NO_2$ ; amido caproic acid, or common leucin  $C_6H_{13}NO_2$ ; amido-valerianic acid  $C_5H_9NO_2$ ; these latter two crystallise together or occur in mixtures in equivalent proportions; they are deposited from boiling solutions of alcohol of 90 per cent.

The matter insoluble in such alcohol may be dissolved in water, and precipitated with mercuric nitrate, at first by itself, later on with the addition of some sodic carbonate to neutralise excess of acid. The mercuric compounds are washed with boiling water, decomposed by hydrothion, decolorised by animal charcoal, and set to crystallise. The crystals are  $C_5H_7NO_3$ , the anhydride of glutaminic acid, termed here glutimic acid; this acid is monobasic. The second crystals, obtained by the aid of mercuric nitrate and alkali, are a mixture of glutamic (three molecules) with asparaginic acid (one molecule), thus:  $3C_5H_9NO_4 + C_4H_7NO_4$ . In another operation a mixture in equivalent proportions of caproic leucin and glutaminic acid,  $C_6H_{11}NO_2 + C_5H_9NO_4 = C_{11}H_{20}N_2O_6$  is obtained.

9. *The volatile body, or essential oil (albuminol).*—This product, obtained in the chemolysis of albumin with baryta, was examined further by Schützenberger and Bourgois (*Bull. Soc. Chim. Par.* [N. S.] 25, 289; and *Ann. Chim. Phys.* 16, 1879, 325). It appears on the top of the ammoniacal distillate in the first condenser, in the shape of a few drops; its quantity is from five to six per mille of the albumin used; it dissolves in the hydrochloric acid when the contents of the condensers are mixed and the mixture is acid. It contains pyrrol, as the following reactions show. With perchloride of iron it gives a dark green reaction and later on a black deposit; with dilute hydrochloric acid and potassic bichromate, it gives a black precipitate; it dissolves in dilute hydrochloric acid and the solution left to itself deposits red brown flocks, which have the composition of *pyrrol-red*.

	Found	Theory of pyrrol-red
C . .	71.75	71.2
H . .	7.50	6.93
N . .	12.02	13.0
O . .	8.88	8.87

There is probably a small quantity of a higher homologue of pyrrol-red present in the flocks, and a body containing sulphur. The red flocks gave 3.1 per cent. of sulphur, while the oil contained from 2.1 to 2.6 per cent. of sulphur.

By fractional distillation, albuminol freed from all ammonia by agitation with a slight excess of dilute sulphuric acid (50 to 60 grms.), can be divided into portions boiling at different temperatures. One boils over between 113° and 120°, a second portion between 120° and 140°, a third between 140° and 180°, and a fourth above 180°.

The first two portions contain from 18 to 17 per cent. of oxygen and 4.5 to 4.1 per cent. of nitrogen, while in the last two portions the oxygen with the sulphur amounts to from 4.5 to 3.7 per cent. only. From these and the other data furnished by elementary analysis it is probable that albuminol is a mixture of two kinds of products; the most volatile part is probably oxygenated, but free from nitrogen; the less volatile part is nitrogenised, but free from oxygen. Now as pyrrol is present (which boils at 133°) it may be allowed that the 4.5 to 4.1 per cent. of nitrogen which are found in the portions of liquid which boil over between 113° and 140° are derived from pyrrol which is mixed with an oxygenated non-nitrogenised substance. The analyses of the first two distillates lead to an empirical formula of  $C_{16}H_{23}NO_3$ , which requires C=69.3; H=8.3; N=5.0; O=17.4. From this view the sulphur found is entirely omitted. If from the foregoing formula the nitrogen is deducted in the form of pyrrol  $C_4H_5N$ , there remains  $C_{12}H_{18}O_3$ , or  $3(C_4H_5O)$ . The portions boiling between 140° and 180° show a composition similar to that of the higher homologues of pyrrol, methyl- and ethyl-pyrrol. They rapidly yield pyrrol-red with dilute hydrochloric acid when exposed to air. The sulphur compound has not been isolated. No trace of indol has been found in this reaction, which is the more to be noted,

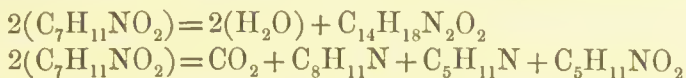


as indol is easily obtained from albuminous matters by chemolysis with fusing potash and by putrefaction with the aid of pancreatic ferment.

10. *Tyroleucin*.—Among the amido acids which Schützenberger (*Compt. rend.* 84, 124) succeeded in isolating when he decomposed as many as ten kilogrammes of albumin with baryta, is one to which he has given the name of tyroleucin. The liquid obtained by the action of baryta upon albumin at 150° is precipitated by carbonic acid, filtered and concentrated; the crystals which separate are, in the main, a mixture of leucin, tyrosin, and butalanin. The syrupy mother-liquor is diluted anew with water, and treated with an appropriate amount of sulphuric acid, to remove the baryta which is not felled by carbonic acid. After filtration and concentration a second crop of crystals is obtained, and after their removal a third. From these second and third crystals tyroleucin is obtained by a great number of fractional crystallisations (from 10 kilos of albumin about 60 or 70 grms.), aided by animal charcoal and basic lead acetate, etc. It has a chalk-like appearance, crystallises in more or less voluminous masses, is soluble in water of 16° in the proportion of 5·3 : 100, more soluble in hot water, very little soluble in cold alcohol of 90 per cent., more soluble in hot alcohol, insoluble in ether. Heated while air is excluded, it begins to fuse at 245° to 250°, and is decomposed, yielding a distillate consisting of water, of the carbonate of a volatile base in a liquid state, and some few crystals; the fluid possesses a strongly alkaline reaction, forms clouds with strong hydrochloric acid, and has a peculiar odour and taste reminding of radish. The walls of the retort in which the distillation was performed are covered with a white snow-like sublimate, and in the bottom of the retort there remains a yellow fluid, which sets into a solid on cooling. When heated with a few drops of nitric acid on platinum, and evaporated, tyroleucin leaves a yellow residue, which becomes orange-coloured with caustic potash. Nitroso-nitrate of mercury, and microscopic examination, prove the complete absence of tyrosin. The elementary composition of tyroleucin is  $C_7H_{11}NO_2$ . The carbonated alkali, contained in the pyrogenetic distillate on neutralisation with hydrochlor, forms a compound, which with



platinic chloride forms a yellow crystalline double-salt of the formula  $\text{PtCl}_4 + 2(\text{ClHC}_8\text{H}_{11}\text{N})$ . From this an oily base can be isolated by distillation with caustic lime; it smells and tastes of radish, and has the composition of collidin. The white sublimate has the properties of butalanin, and the non-volatile residue, which solidifies on cooling, and is soluble in alcohol, has the formula  $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_2$ . The pyrolytic reaction is therefore probably also synthetic or polymerising, and produces the ascertained results, according to the following equations:



In the crystalline deposits which furnish tyroleucin much leucein and butalanin are contained. Leucein may therefore possibly be a compound in equivalents of tyroleucin and butalanin.



Leucein is indeed stated to behave like tyroleucin on pyrolysis, but to yield more butalanin. On the other hand, tyroleucin may be considered as a compound of butalanin  $\text{C}_5\text{H}_{11}\text{NO}_2$  with a body of the formula  $\text{C}_9\text{H}_{11}\text{NO}_2$ , which differs from tyrosin only by an atom of oxygen which it contains less. When tyrosin is heated under the same conditions as those effecting the changes of tyroleucin above described, it splits up into carbonic acid and an oxygenated base of the formula  $\text{C}_8\text{H}_{11}\text{NO}$ , which differs from collidin only by containing an atom of oxygen not present in collidin.

11. *Protoconia*.—Closely related to the bodies described in the foregoing is probably a volatile ammonium base from animal albuminous matter, discovered by Thudichum in 1869 ('Rep. on Researches, etc.,' in the *Twelfth Report of the Medical Officer of the Privy Council*, 1869, p. 257). It was obtained by chemolysis in dilute sulphuric acid of albuminous constituents of brains, lungs, livers, kidneys, and muscles from human subjects. The products were distilled with excess of caustic lime in water, and yielded a distillate containing much ammonia and a compound ammonium base. The bases were first crystallised as chlorides, then redistilled, and transformed a second time into chlorides; again distilled over potash, they were transformed into sulphates, and from these the compound

base was extracted by absolute alcohol, while the great bulk of the product, ammoniac sulphate, remained undissolved. The sulphate of the new base, after distillation of the alcohol, was distilled with soda, the distillate transformed into chloride, and set to crystallise. Several crystallisations were isolated, and the crystals combined with platinic chloride; the double-salts were again subjected to fractional crystallisation, and the purest products analysed. The quantitations led to  $\text{PtCl}_4 + 2(\text{HClC}_7\text{H}_{11}\text{N})$ . The hydrochlorate was  $\text{C}_7\text{H}_{11}\text{NHCl}$ , and the free base  $\text{C}_7\text{H}_{11}\text{N}$ . Thudichum named the base *protoconia*, from its similarity in composition, smell, and other properties to conia (or coniine), the volatile base from hemlock.

Horn, which yields other products of the chemolysis of albuminous substances with facility, did not give any protoconia when treated, even in quantities up to 20 kilogrammes, like the albuminous substances mentioned.

Protoconia differs from tyroleucin only by  $\text{O}_2$ , but it could not be derived from it by the same process as that which takes place in the pyrogenetic process of Schützenberger. It is more probably a direct product of the first sulphuric acid chemolysis, and has not as yet been obtained from other than human albuminous matters.

12. *Chemolysis of the collagenous substances by baryta hydrate.*—This subject was further investigated by Schützenberger and Bourgois (*Compt. rend.* 82, 262) upon isinglass, ossein, gelatin, and chondrin from the cartilages of the calf. There were obtained from 100 parts—

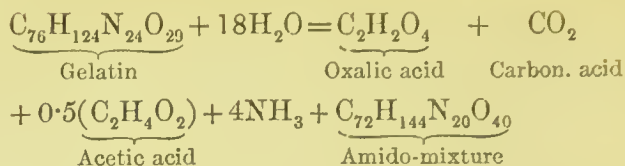
		Isinglass	Ossein	Gelatin	Chondrin
Nitrogen as ammonia . . .		3.48	3.35	2.8	2.88
Oxalic acid . . .		4.1	3.62	3.3	4.2
Carbonic acid . . .		2.7	3.1	2.72	2.45
Acetic acid . . .		1.7	1.44	1.5	4.69
Elements of amido-mixture	C .	44.83	46.48	45.16	46.7
	H .	7.37	7.45	7.36	7.07
	N .	14.44	14.10	14.30	11.7
	O .	33.36	32.23	33.18	34.63

From these analyses the following formulæ are derived, nitrogen assumed as unit;—

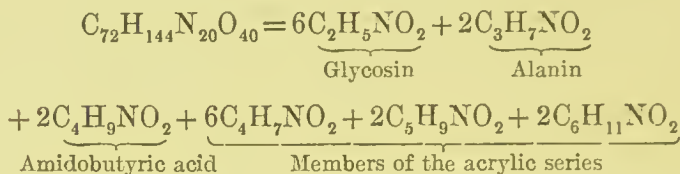
Isinglass	= $C_{3.62}$	$H_{7.146}$	N	$O_{2.220}$
Ossein	= $C_{3.88}$	$H_{7.25}$	N	$O_2$
Gelatin	= $C_{3.66}$	$H_{7.20}$	N	$O_{2.08}$
Chondrin	= $C_{4.676}$	$H_{8.49}$	N	$O_{2.57}$

As with the albumins proper and with fibroin, so with the above substances, the nitrogen evolved (by baryta chemolysis) in the form of ammonia on the one hand, and the carbonic and oxalic acid on the other hand, stand in such proportions to each other that the simultaneous formation of these three bodies can be referred to hydration of urea and oxamide. For gelatin and chondrin the harmony is nearly perfect; isinglass and ossein give an excess of nitrogen found amounting to 0.3 to 0.25 over the quantity calculated from the weight of carbonic and oxalic acids.

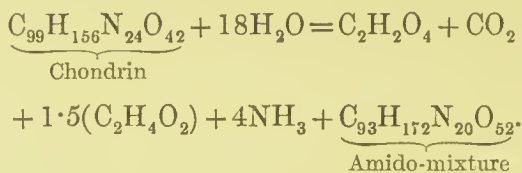
The analysis of the amido-mixture shows that its composition is identical, or nearly so, for isinglass, ossein, and gelatin. The proportion between the atoms of nitrogen and oxygen is nearly as 1 : 2; it may therefore be expected that only members of the two series  $C_nH_{2n+1}NO_2$ , and  $C_nH_{2n-1}NO_2$  are present. The proportion between the atoms of carbon and hydrogen is as 1 : 2; the nitrogen is therefore almost accurately divided between members of the series  $C_nH_{2n+1}NO_2$ , and  $C_nH_{2n-1}NO_2$ . Chondrin shows peculiarities in the amount of acetic acid which it yields, which is threefold that of the other substances; further in this that the number of atoms of nitrogen are to those of oxygen in the proportion of 1 : 2.57, which indicates the probable presence of compounds of the series  $C_nH_{2n-1}NO_4$  in the amido-mixture; further, that the proportion between the atoms of carbon and hydrogen is nearly as  $n : 2n-1$ , from which it follows that the members of the two series  $C_nH_{2n-1}NO_2$ , and  $C_nH_{2n-1}NO_4$  prevail. The probable composition of the amido-mixture from isinglass, ossein, and gelatin is therefore: glycosin 20 to 25 per cent.; alanin,  $C_3H_7NO_2$ ; amidobutyric acid  $C_4H_9NO_2$ ; traces of glutaminic acid; members of the series  $C_nH_{2n-1}NO_2$ , with  $n=4, 5$ , and 6 more than 50 per cent. When these results are compared with the original composition of isinglass, ossein, and gelatin, the following formula for the reaction which they undergo with baryta hydrate may be written:—



The amido-mixture decomposes in the following manner :—



The amido-mixture of chondrin is almost destitute of glycosin ; it contains the acids of the series  $C_nH_{2n-1}NO_4$ , alanin, amidobutyric acid, and the members of the acrylic series  $C_4H_7NO_2$  and  $C_5H_9NO_2$ . For its decomposition the following formula may be written :—



In these compounds the number of molecules of water fixed during chemolysis is smaller than the number of atoms of nitrogen in the products.

13. *Chemolysis of Fibroin (Silk).*—This body was subjected to chemolysis by baryta hydrate by Schützenberger and Bourgois (*Bull. Soc. Chim. Par.* [N.S.] 25, 1), with the result that it yielded ammonia, oxalic and acetic acid, and a mixture of amido compounds. 100 parts of fibroin gave nitrogen in form of ammonia 2 per cent. ; mixture of barytic carbonate and oxalate 18 per cent. ; acetic acid in very small quantity ; mixture of glycosin and alanin in equivalents 60 per cent. ; amidobenzoic acid 10 per cent. ; tyrosin 9.5 to 10 per cent. ; amides of the series  $C_nH_{2n-1}NO_2$ ,  $C_4H_7NO_2$  preponderating, about 20 per cent. The elementary composition of the amido-mixture compared with that of the fibroin shows that the amount of water taken up during the reaction stands to the nitrogen of the fibroin in the proportion of  $H_2O : N$ .



The hair of the alpaca is of a similar chemical constitution. The fixed residue contains  $C=40.6$ ,  $H=7.3$ ,  $N=15.0$ . Ammonia and non-nitrogenised acids are obtained in much lesser quantities than from hair of other animals or sheep's wool.

14. *Chemolysis of sheep's wool and human hair.*—P. Schützenberger (*Compt. rend.* 86, 1878, 767) subjected sheep's wool to the influence of an equal weight of baryta hydrate, mixed with water amounting to three or four times the weight of the wool. It yielded the general products of the chemolysis of the albuminous substances in the following proportions. 100 grms. gave—

Nitrogen in the form of ammonia	. 5.2 to 5.3
Carbonic acid, separated as baryum salt .	4.24 to 4.3
Oxalic acid . . . . .	5.68 to 5.77
Acetic acid, estimated volumetrically in the distillate, obtained from the mix- ture after elimination of the baryta by carbonic and sulphuric acid .	3.18 to 3.20
Pyrrol and other volatile products .	1 to 1.5
Composition of the fixed residue or mix- ture of the amidated substances pro- duced by cleavage : $C=47.85$ ; $H=7.67$ ; $N=12.63$ .	

A specimen of Australian wool, freed from fat, gave the same quantities of ammonia, acetic, oxalic, and carbonic acid; the fixed residue gave on elementary analysis  $C=48.03$  ;  $H=8.24$  ;  $N=12.9$ . It seems therefore that specimens of wool of different origin differ a little in composition. The fixed residue from Australian wool has the same composition as that derived from albumin; the fixed residue from the first specimen of wool is a little different.

The fixed residue consists exclusively of the following amidated bodies: caproic leucin,  $C_6H_{13}NO_2$ ; caproic leucein,  $C_6H_{11}NO_2$ , =12 to 15 per cent.; tyrosin,  $C_9H_{11}NO_3$ =3.2 per cent.; butyric leucin,  $C_4H_9NO_2$ , and valeric leucin,  $C_5H_{11}NO_2$ , the former prevailing; propionic leucin,  $C_3H_7NO_2$ ; butyric and valeric leuceins,  $C_5H_7NO_2$  or  $2(C_4H_7NO_2)$ ,  $C_4H_5NO_2$  or



$2(\text{C}_5\text{H}_9\text{NO}_2)$ ; glucoprotein intermediate between the leucins and the leuceins,  $\text{C}_8\text{H}_{16}\text{N}_2\text{O}_4$

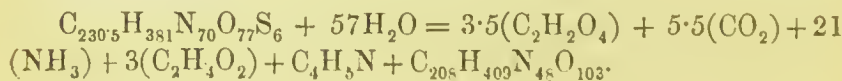
Human hair gives the same kind of fixed residue as wool, but it yields larger quantities of carbonic, oxalic, and acetic acid.

A small quantity of a syrupy acid (*lanophanic acid*) was also obtained, which had a freely acid reaction and gave a crystallisable silversalt. This acid remains in the barytic carbonate, when the first liquid is treated with carbonic acid. The barytic carbonate is decomposed with sulphuric acid, and the filtered liquid is concentrated. Analyses of the free acid and of its silversalt lead to the formula  $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_6$ , the silversalt being  $\text{C}_{10}\text{H}_{14}\text{Ag}_2\text{N}_2\text{O}_6$ . This acid is also found amongst the decomposition products of albumin.

The acid is almost identical in composition with paraphanic acid,  $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_6$ , the silversalt of which is  $\text{C}_{11}\text{H}_{14}\text{Ag}_2\text{N}_2\text{O}_6$ ; it differs from kryptophanic acid,  $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_{10}$ , by the latter acid containing  $\text{H}_2\text{O}_4$  more. There is evidently a very close relationship between the 'lanophanic' acid from wool and albumin, and the extractive acids contained in urine. (Compare Report for 1878, App. B, No. 2, pp. 325 and 331.)

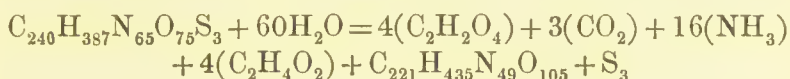
15. *Probable Formulae of the higher Organoplastic Substances.*—On attempting to give a formula for the decomposition of wool or of albumen, which should contain an entire molecule of the body formed by chemolysis in the smallest quantity, one finds the atomic weight of wool or of albumin to be very high.

Wool contains, according to the published analyses,  $\text{C}=50\cdot0$ ;  $\text{H}=7\cdot0$ ;  $\text{N}=17\cdot7$ ;  $\text{O}=22\cdot0$ ;  $\text{S}=3\cdot1$ . This leads to the smallest formula of  $\text{C}_{3\cdot295}\text{H}_{5\cdot46}\text{N}_{1\cdot011}\text{S}_{0\cdot09}$ . Now as 100 parts of wool give at the utmost 3·2 parts of tyrosin, wool must have a molecular weight of at least 5,500 in order to give the opportunity for the formation of a single molecule of tyrosin ( $=181$ ); for  $\frac{181}{55\cdot00} = 3\cdot2$ . The smallest formula derived from the percentic composition of wool just given, when multiplied by 70, leads to  $\text{C}_{230\cdot5}\text{H}_{381}\text{N}_{70}\text{O}_{77}\text{S}_6$ , and this formula is decomposed by the chemolytic process in the sense of the equation



If from the last formula, which expresses the composition of the amidated mixture, we deduct a molecule of tyrosin  $C_9H_{11}NO_3$ , the rest  $C_{199}H_{398}N_{47}O_{100}$  is, as in the case of albumin, a value of the formula  $C_nH_{2n}N_2O_4$ , with a slight excess of oxygen;  $n=8.46$  for wool;  $n=8.8$  for albumin. The expressions are therefore homologous for wool and for albumin, and the difference is due to one in the quantity of leucin.

The equation for the decomposition of albumin (of which the empirical formula is multiplied by a convenient factor to attain a molecule of tyrosin) is the following:—



In both cases the free ammonia is a function of the three non-nitrogenised acids, every molecule of carbonic and oxalic acid corresponding to  $2NH_3$ , and every molecule of acetic acid corresponding to  $NH_3$ . The partition of the rest of the nitrogen is effected equally between the bodies of the general formula  $C_nH_{2n+1}NO_2$  on the one, and the bodies of the formulæ  $C_nH_{2n-1}NO_2$  and  $C_nH_{2n-1}N\left\{\begin{smallmatrix} O_3 \\ O_4 \end{smallmatrix}\right\}$  on the other hand.

The above formula for albumin gives a molecular weight of 5743, and the theory of its elementary composition accords with experience as follows.

	Theory	Found
C . .	52.62	52.57
H . .	7.07	7.16
N . .	16.62	16.6
O . .	21.94	21.8
S . .	1.75	1.8

16. *List of terminal cleavage products, mostly crystalloids obtained by the chemolysis of albuminous and colloigenous substances—*

Carbon tetrahydride (marsh gas)	. CH <sub>4</sub>
Ammonia . . .	. NH <sub>3</sub>
Carbonic acid . . .	. CO <sub>2</sub>
Oxalic acid . . .	. C <sub>2</sub> H <sub>4</sub> O <sub>4</sub>
Sulphurous acid . . .	. SO <sub>2</sub>

Indol . . . . .	$C_{16}H_{14}N_2$
Skatol . . . . .	$C_{10}H_{11}N$
Pyrrol . . . . .	$C_4H_5N$
Epipyrrol . . . . .	$C_5H_7N (?)$
Acetic acid . . . . .	$C_2H_4O_2$
Tyrosin . . . . .	$C_9H_{11}NO_2$
Amido-cænanthic acid . . . . .	$C_7H_{15}NO_2$
Tyroleucin . . . . .	$C_7H_{11}NO_2$
Protoconia . . . . .	$C_7H_{11}N$
Amido-benzoic acid . . . . .	$C_7H_7NO_2$
Amido-caproic acid (leucin). . . . .	$C_6H_{13}NO_2$
Amido-valerianic acid . . . . .	$C_5H_{11}NO_2$
Amido-butyric acid . . . . .	$C_4H_9NO_2$
Amido-propionic acid (alanin) . . . . .	$C_3H_7NO_2$
Amido-oxypropionic acid (serin) . . . . .	$C_3H_7NO_3$
Amido-acetic acid (glycosin) . . . . .	$C_2H_5NO_2$
Caprono-leucein . . . . .	$C_6H_{11}NO_2 (?)$
Valeriano-leucein . . . . .	$C_5H_9NO_2$
Butyro-leucein . . . . .	$C_4H_7NO_2$
Glutamic acid . . . . .	$C_5H_9NO_4$
Glutimic acid . . . . .	$C_5H_7NO_3$
Asparaginic acid . . . . .	$C_4H_7NO_4$
Hypoxanthin . . . . .	$C_5H_4N_4O$
Dextrin . . . . .	$n(C_6H_{12}O_6)$
Cellulinamide . . . . .	$n(C_6H_{13}NO_6)$
Phenol . . . . .	$C_6H_6O$
Lanophanic acid . . . . .	$C_{10}H_{16}N_2O_6$
Lactic acid . . . . .	$C_3H_6O_3$
Succinic acid . . . . .	$C_4H_4O_3H_2O$

## III.

ON THE ACTION AND PRODUCTS OF THE STARCH-TRANSFORMING FERMENTS, DIASTAS, PTYALIN, PAN-CREATIN, ACIDS, AND VARIOUS MATTERS DERIVED FROM ALBUMINOUS SUBSTANCES. (*Summary.*)

THE scientific consideration of the malting of barley dates from the end of last century, and may be said to have begun with the observation of Cruikshank (Scherer's *Allg. Journ. d. Chem.*, Leipzig, 1798, vol. 1), according to which the sprouting of moistened barley, and consequently the formation within its substance of sugar, do only take place in the presence of an ample supply of oxygen. This observation induced Cruikshank to attribute the formation of sugar to an oxidation of the vegetable mucilage and the starch-like components of the seed. In 1811, however, it was discovered by Kirchhof (Schweigger's *Journ.* 14, 389) that the transformation of starch within sprouting grain is effected by an albuminous body contained in the seeds; he imitated the process by placing gluten, from wheat-flour, washed free from starch, in contact with potato-starch, made into paste with hot water, and observed that in a few hours the paste became fluid, and contained sugar. On applying powdered malt to a paste made from potato-starch, Kirchhof found that it effected the transformation in a shorter time than gluten; but he continued to attribute the action to gluten in both cases, while admitting that its peculiar power was much increased in the growing seeds by the process of germination. At the same time this chemist discovered that boiling dilute mineral acids had a power similar to that of malt or gluten, of transforming starch into sugar.

The mode in which starch is digested in the animal body had long been an object of sterile inquiry on the part of physio-

logists, when it was discovered by Leuchs (Kastner's *Archiv*, 1831) that saliva had the same power as malt or gluten, of producing sugar and dextrin in starch-paste made with the aid of water and heat. This was confirmed by Schwann (*Poggend. Ann.* 38, 358). The attention of chemists now became more generally directed upon the subject, and many endeavours were made to define and isolate the body to which this remarkable metamorphosis was due. Th. de Saussure (*Poggend. Ann.* 32, 1834, 194) extracted three different bodies from crude gluten, which were distinguished from each other by differing solubilities in alcohol, and termed them respectively (vegetal) albumin, gluten, and mucin; of these the last, mucin, possessed in the highest degree the faculty to dissolve starch-paste, and transform it into sugar, while the first two possessed it in a much lesser degree. Payen and Persoz now described a method by which the starch-transforming substance could be isolated; malt was digested with water, and the solution mixed with alcohol, whereby a precipitate containing the active body was produced. The greater part of the precipitate was found to be again soluble in water, and then to exhibit the starch-transforming power in an eminent degree. The part of the alcohol precipitate insoluble in water was found to be albumin. The soluble ferment, when dried at a low temperature, was amorphous, yellow and translucent; soluble in water and weak spirit; when heated in the moist state or in solution to 75° C. it lost its starch-transforming power; it also became inert when kept for a longer period in the moist state or in solution. In the fresh state its power was so great that one part effected the solution of 2,000 parts of potato-starch in a few minutes. Payen and Persoz termed this substance, which they considered as a chemical individual endowed with specific properties, diastas. This name was chosen to express the hypothesis which they had formed concerning the manner in which the starch granules are transformed, namely, by bursting of their shells, and pouring out of their supposed gumlike contents. The name, still generally used, has survived the hypothesis, now generally abandoned, to which it owed its origin. Payen and Persoz found diastas to be present not only in malt, but also in growing seeds of oat and wheat (*Ann. Chim.* 53, 73; 56, 337), of maize and



rice (*Ann. Chim.* 60, 1835, 441), in the sprouts of potatoes, and in the buds of *ailanthus glandulosa*.

The theory of ferments thus becoming more and more developed, Mialhe (*Mém. sur la Digestion et l'Assimilat. des Mat. amyloïdes*, 1845), following up the discovery of Leuchs, separated a body from saliva which was the bearer of the sugar-forming action upon starch; it was termed, after Berzelius, ptyalin, and its analogy to, or even identity with, diastas, was allowed or maintained. Bouchardat (*Ann. Chim.* [3,] 14, 1485, 60) found that various matters of animal origin, albumin, gelatin, fresh fibrin, still more albuminous matters in a state of decomposition, such as putrid flesh, putrid gluten from plants, and others, were able to dissolve starch and transform it partially into sugar much like diastas. Sandras and Bouchardat (*Compt. rend.* 20, 143) demonstrated the presence in the pancreas of a ferment possessing a power similar to that of ptyalin. The natural secretion of this gland, or even an infusion of the minced organ, was found to produce sugar in starch-paste with great rapidity. These data, with the occasional inquiries of Magendie, Lassaigne, Barreswill, Bernard, Hensen, Wright, Schiff and others, showing that bile, decomposed urine, blood serum, blood, brain matter, heart, muscle, lung, liver, kidney, were all able to exert a function upon starch more or less like that of diastas, led to the doctrine of the universal presence in vegetal and animal parts of starch-transforming ferments, a doctrine of which Mulder became the principal systematic expositor (*Chem. des Bieres*, p. 215). He was of opinion that there would hardly be found a vegetal juice destitute of the power to transform starch into dextrin and sugar, and believed the same power to be inherent in all animal fluids and solids, when placed under the necessary conditions.

After Von Wittich had discovered a new method for concentrating certain shapeless animal ferments he also applied it for the purpose of isolating a diastatic ferment from the animal matters known to exhibit the function (*Arch. Physiol.*, 3, 1870, 339). He obtained active matters in small quantity from blood, blood serum, kidneys, brain, mucous membrane of stomach and intestines, but arrived incidentally, so to say, at the belief that this ferment was not exclusively a

specific product of the life of certain cells in the parenchyma of plants, but also took its origin in the general chemistry of the body. Lepine (*Ber. K. Sächs. Ges. d. Wissensch.*, October 1870) found a diastatic action in nearly all tissues of the body, but not in the crystalline lens. After four days' keeping the lens also exhibited the action, from which he drew the conclusion that the fermenting action is developed in the organs by post-mortem change; as already observed by Bouchardat as quoted above and by Bernard upon washed fibrin (*Leçons de Physiol. expér.* 2, 1856. Seegen and Kratschmer (*Arch. Physiol.* 14, 1877, 593) have made similar observations. Even when they boiled and washed brain or muscles, these tissues regained a starch-transforming action already after a few hours; pure serum albumin, egg albumin, casein, and fibrin acted in the same manner. But the action was slow and feeble as compared with that of ptyalin and pancreatin. They believed that the liver contains a specific diastatic ferment, but were unable to extract it by the alcohol and glycerin method of Von Wittich (*Arch. Physiol.* 7, 1873, 28). In a later publication Seegen seems to have abandoned this opinion (*Arch. Physiol.* 19, 1879, 106). The ground-up liver, after having been hardened with alcohol, yielded to glycerin a mixture of glycogen and diastatic ferment. In this solution the ferment remained inactive, as long as water was not added; when this was added the formation of sugar began at once. This mixture had already been extracted by Epstein and Müller (*Ber. Deutsch. Chem. Ges.* 8, Heft 4). The return of the diastatic action to boiled liver magma on standing had been already observed by Abeles (*Med. Jahrb.* 1876, Heft 2).

Concerning the general characters of these ferments Von Wittich found (after Schönbein, *Zeitsch. f. Biologie*, 4, 1868, *Journ. Pract. Chem.*, 106, 1869, 257) that they show an energetic action upon hydrogen peroxide, and retain this as well as their starch-transforming action at temperatures from 60° to 80° C., at which the albuminous substances coagulate.

Von Gorup Bcsanez (*Ber. Deutsch. Chem. Ges.*, 1874, p. 1478, and 1875, p. 1510) in extracting diastatic ferment by Von Wittich's process from vetches, hemp and linseed obtained mixed with this a ferment which had the power to dissolve and

transform into peptones fibrin and coagulated white of egg. This observation was likely to shake the belief in the peculiarity of many of the so-called specific ferments, and made the digesting power of the so-called insectivorous plants a particular case of a widely diffused faculty of vegetals.

The distribution of starch-transforming ferments in plants has quite lately been studied by J. Baranetzky (*Die Stärkeumbildenden Fermente in den Pflanzen*, Leipzig, 1878, 8vo. pp. 64 and plate). He made concentrated watery extracts, of which he added from 0.5 to 1 c.c. to 3 to 4 c.c. of a solution of potato-starch prepared by boiling, and containing not above 1 per cent. of dry starch. The presence of the ferment was recognised by the liquefaction of the paste and its transformation into a perfectly clear liquid. This criterion is more certain according to this author, than the appearance of sugar, because as he says, the starch may be dissolved without, or at all events before, any sugar is formed. He did not operate with pure ferments, nor did he investigate the processes on any larger scale than in test-tubes; he made no analyses of the products, and no quantitative tests except some reductions with standard copper solution, so that his results have no stoichiometric backbone. But they are conceived in a philosophical spirit, and extend our knowledge of the distribution of the diastatic power in the vegetal world. The following is a list of parts of vegetals in which he found the diastatic or starch-transforming power.

*Seeds containing starch.*—*Phaseolus multiflorus* (germinating). *Vicia faba*, sprouting in the dark, etiolated; cotyledons. *Pisum sativum*: the crushed hard seeds dissolve starch in 20 hours; seeds germinating in light effect the same work in 30 minutes. *Polygonum fagopyrum*: growing seeds. *Mirabilis Jalapa*: the seeds dissolve paste; the germinated seeds have no greater action. *Aesculus Hippocastanum*: slow action, whether germinating or not. *Quercus pedunculata*: no action, whether germinating or not. (Diasas forms an insoluble compound with tannin or tannic acid.—Dubrunfaut 1868.)

*Tubers containing starch.*—*Potatoes*, growing shoots; *Gesneria barbata*, sprouting; *Dioscorea Batatas*, tubers with prouts more than a metre in length: the extract, which was

mucilaginous, dissolved starch in 30 minutes. *Iris germanica*, with green leaves 10 to 15 c. long, same effect.

*Stalks and leaves.*—*Phaseolus multiflorus*; *Pisum sativum*; *Vicia faba*: the extracts dissolve the starch in from 3 to 20 hours. *Daucus carota* and *Brassica Rapa*; shoots from roots dissolve the starch in from 30 to 90 minutes. *Eriobotrya japonica* (leaves); *Acanthus cordifolia* (leaves); *Echium giganteum* (leaves); *Tradescantia zebrina* (leaves); *Veltheimia viridiflora* (leaves) dissolved the paste in from 20 to 48 hours. *Rhizomes free from starch.* *Daucus carota* contains much copper-reducing sugar, and cane-sugar; transforms starch in 30 minutes. *Brassica Rapa* contains also a reducing sugar; transforms starch in 20 minutes.

The difference in the time required for full action is ascribed to differences in the concentration only.

Few inquirers were induced by this observation to distinguish between a ferment, which transforms the starch in plants into a soluble nutriment (not necessarily sugar or dextrin) without requiring the aid of heat for the fluidification of the starch, and another which does not act upon starch in granules as obtained from vegetable parts, but requires the granules to be previously hydrated and disintegrated by water and heat. In an article on the process of digestion in animals and vegetals (*Revue Scientif.* 1873, 515) Bernard compared the action of the starch-transforming ferments with each other. He impressed upon his readers the fact that their action appears in seeds of cereals with the earliest symptoms of germination, in potatoes in spring even before they have been committed to earth for regeneration. In this as in most other discussions of the subject it was assumed that the ferments which liquefy starch in living plants, and maybe transform it into nutriment for new growth, or even into sugar, are the same as diastas, the ferment extracted from malt, which transforms boiled starch into maltose and dextrin, but has either no effect, or only a very slow action upon unboiled or unhydrated amylon. This assumed identity is however not only not proved but directly contradicted by the data. The data indeed lead us to believe, that if starch is transformed into sugar during the germinating process of barley, it is probably not by the agency of diastas



alone that this change is effected. And it further follows as a result of the consideration, that there must be a difference between the starch-transforming ferments in animals, which fluidify, hydrate and split up starch without the previous application to it of heat, and those in malt, which can only, or at least mainly (time being a condition of the comparison) transform starch previously so to say opened up by heat. Guérin-Varry (*Ann. Chim.* 60 (1835) 32) observed that potato-starch suspended in malt extract, and allowed to stand during 63 days at 20° to 26° underwent no change that could be observed either chemically or microscopically. At 54° to 55° only did any diastatic action begin to manifest itself, a temperature at which the starch granules began to swell and burst. Schlossberger (*Organ. Chem.* 1857, 121) made a similar experience and came to the conclusion, that the solution of starch in growing seeds is effected by a ferment different from the diastas which can be isolated from the seeds.

Mulder was of an opposite opinion, and explained (*Chem. des Bieres*, p. 222) the difference as caused by difference in the quantity or concentration of the ferment. Baranetzky (l.c. 38) explains that these results, which opposed the idea of the unity of the starch-transforming ferment, were due to the accident that the experiments were all made upon potato-starch, which is one of the most resistant to the influence of ferments. Wheat or buckwheat-starch however is attacked by ferments at the ordinary temperature in the same manner as by ferments in the seeds. The experiments were made each with 2 to 3 centigrammes of pure starch in watch-glasses: the ferments consisted of the watery solution of precipitates produced by alcohol in vegetal extracts. The starches examined came from *Polygonum fagopyrum*, *Phaseolus multiflorus*, *Mirabilis Jalapa*, *Quercus pedunculata*, *Aesculus Hippocastanum*, potatoes, wheat and rice. The microscopically visible changes which the granules undergo have been represented by Baranetzky on a plate with many figures. In now passing to a more particular examination of the products which starch yields under the influence of various ferments akin to diastas or of diastas itself, it must be stated at once that our main attention will be directed upon the sugar-like products, and that the con-



sideration of the dextrin-like products, and of the question whether they are always, or in fact, transition-products, will be treated of only to such an extent as may be necessary to define its present position.

Most sweet-tasting substances occurring in natural edible vegetal products were believed nearly to the end of the last century to be cane-sugar. The distinction between cane-sugar and grape-sugar and the identification of the latter as a peculiar body were not effected until 1792, by Lowitz (*Crell's Chem. Ann.* 1, 218 and 345) and Proust (*Journ. de Phys. et de Chim.* 63, 257 ; 69, 428 ; *Ann. Chim.* 57, 131 and 225). Many years later Thénard and Dupuytren (*Ann. Chim.* 44, 45) discovered the identity of diabetic and grape-sugar. Many authors afterwards enlarged our knowledge of these transformations, particularly after Biot had taught a new means of diagnosing the new products from each other by their varying influence upon polarised light. But no author contributed during almost forty years, beginning from 1823, more or better new knowledge concerning glucose, and particularly the transformation-products of starch by diastas, than Dubrunfaut. In 1847 (*Ann. Chim.* 21, 178) he prepared the crystallised sugar obtained by the malt ferment, and observing it to show reactions differing from those of ordinary grape-sugar or glucose he submitted it to Biot, who found that its influence upon polarised light was much greater than that of an equal weight of grape-sugar (*Compt. rend.* 15, 710 ; 42, 351). Forthwith Dubrunfaut announced its peculiarity, showed that it was transformed into grape-sugar by boiling with dilute acids, that it fermented with yeast like grape-sugar, and termed it maltose.

This most important discovery was either altogether ignored or doubted, or even strongly opposed, by almost all chemical authors and inquirers on the subject. This misguidance has affected a vast number of statistical researches in which the copper-solution test or the polarisation test have been relied upon as quantitative indices for grape-sugar.

O'Sullivan (*Journ. Chem. Soc.* 25, 1872, 579) repeated the experiments of Musculus (*Ann. Chim.* [3] 55, 203), of Payen (*Ann. Chem.* [4] 4, 286), and of Schwarzer (*Journ. pract. Chem.* [2] 1, 212), in none of which any regard had been had

to the discovery of Dubrunfaut, and obtained results which, while agreeing only partially with those of the chemists named and quoted, entirely confirmed those of Dubrunfaut as regards maltose.

*Mode of preparing maltose or barley sugar.*—100 grms. of air-dried starch are mixed with 300 c.c. of water at 40°, and the mixture is well stirred to diffuse as completely as possible the starch granules through the liquid, and then poured with continual stirring into 2 litres of boiling water. The paste is cooled to 40°, the extract, prepared with cold water, from 20 grms. of pale malt added to it, and the mixture kept at a temperature of from 40° to 45° during three hours, or until the iodine test shows that all starch has disappeared. It is then boiled for some time, cooled, filtered, and the filtrate evaporated at 80° to 300 c.c. The solid matter in solution, which is a mixture calculated from the specific gravity (taking 10 grms. in 100 c.c. to be represented by 1.0385) has a specific rotatory power  $[\alpha] = +170^\circ$ , and is not changed either by boiling or evaporation. The syrup is boiled for a short time with two litres of alcohol, sp. gr. 0.820; on cooling the clear solution is decanted from the undissolved syrup, and put aside in a stoppered flask. At the end of about a week the sides of the vessel are found covered with a crystalline crust of pure barley sugar or maltose.

Musculus and Gruber have altered the foregoing procedure a little by adding ether to the alcoholic solution of the maltose at intervals, and removing several successive crystallisations. In this way four litres of alcoholic solution receive successively one litre, two litres, two litres, two litres--altogether seven litres of ether. Each addition produces a precipitate or crystallisation on long standing, but only the precipitates after the third and fourth addition are pure maltose.

*Properties of maltose.*—This sugar forms white crystals, which are very soluble in water, but less soluble in alcohol than grape-sugar. Dried at 100° in a current of dry air, it has a specific rotatory power of  $[\alpha] =$  from  $+149$  to  $+150^\circ$ . (Dubrunfaut had given  $+150.6$ , or three times the rotatory power of dextrose,  $+55.2 \times 3$ ). The rotatory power of a recently prepared solution is not stronger than that of the same solution,

after the lapse of several hours. Maltose does not therefore exhibit the so-called bi-rotation of dextro-glucose, that is to say, the peculiarity of rotating the ray of polarised light about twice as much immediately after solution as after some hours of standing. Maltose expands in crystallising, and in this respect resembles cerebrose. By boiling with dilute sulphuric acid it is converted into dextrose. (Starch on being boiled with dilute sulphuric acid also yields at first maltose, and the formation of dextrose is only a secondary reaction between the acid and the maltose.) Maltose is not so easily altered by watery solutions of caustic alkalies as dextrose. Under the influence of yeast it is decomposed into alcohol and carbonic acid, like dextrose, but without transforming into that body (as cane-sugar does previously to fermenting). According to Dubrunfaut, when a mixture of dextrose and maltose is fermented, both kinds of sugar are decomposed simultaneously, not one before the other; according to O'Sullivan, however, the whole of the dextrose disappears before the maltose is touched. Maltose is less oxydisable than dextrose, so that while a given weight of dextrose will reduce 100 parts of Fehling's fluid, the same weight of maltose will only reduce 65 to 67 parts. This reaction, although always the same apparently, does not admit of any stoichiometric interpretation. Two molecules of dextrose take up 5 molecules of oxygen from an alkaline copper solution, but two molecules of maltose take up 3.25 molecules of oxygen, a number which does not admit of representation by a simple equation. By elementary analysis of maltose the following data have been obtained :—

Theory		O'Sullivan found		Theory		Hans Meyer found
12 C	42.10	41.97	42.02	12 C	40.00	39.93
22 H	6.43	6.48	6.50	24 H	6.67	6.72
11 O	51.47	—	—	12 O	53.33	—
<hr/>						
100.00						

These lead to the formula of a sugar isomeric with cane-sugar or milk-sugar,  $C_{12}H_{22}O_{11}$ . The needle-shaped crystals of this body, before drying at 100, contain a molecule of water of

crystallisation, and are therefore  $C_{12}H_{22}O_{11} + H_2O$ . O'Sullivan analysed barley sugar dried at  $100^\circ$  in a current of air; Hans Meyer analysed crystallised sugar, produced by Musculus and von Mering, which had stood during four days over phosphoric acid, been heated during 10 hours at  $80^\circ C.$ , and then been kept for several days over sulphuric acid. This body heated for a longer time to  $115^\circ$ , lost 4.71 per cent.  $H_2O$ , while the theoretical loss should have been 5 per cent.

Maltose does not reduce a boiling solution of neutral cupric acetate to which a very little acetic acid has been added. (Barfoed's reagent.) This reagent is also not affected by sugar of milk, but is reduced by glucose after short warming. (*Zeitschr. Analyt. Chem.* 12, 27; *Chem. Centr.bl.* 1873, 357.)

When maltose is dissolved in strong alcohol, and alcoholic solution of potash is added as long as a precipitate is produced, maltose-potash is obtained as a precipitate insoluble in alcohol. After drying in vacuo maltose-potash is white and chalky, and very light, and therefore differs greatly in physical appearance from the glucose-potash, which is a yellow varnish-like solid. Maltose in alkaline solution reduces the salts of bismuth, gold, silver, and mercury.

*Second product of the diastatic zymolysis of starch; dextrin.*—The precipitate obtained by the addition of alcohol to the concentrated solution of starch decomposed by diastas, may be extracted by alcohol, in which it is insoluble and remains as a white waxy mass. It is purified from sugar by dissolving it in water, and reprecipitating it with alcohol, and repeating this process ten to fifteen times; in this state it has a reducing power equal to (in different specimens) from 8 to 12 per cent. of glucose, and a specific rotatory power of  $[\alpha] = +204^\circ$  to  $205^\circ$ . The same substance is obtained, whether starch is treated with diastas or sulphuric or oxalic acid. When this substance is dissolved in water, so as to give a solution of sp. gr. 1.090, and at  $20^\circ C.$  treated with yeast amounting to 2 per cent. of the solid matter in solution, it is changed so as to lose its reducing power over cupric solution almost entirely, only from 0.80 to 2.20 per cent. of its weight of glucose being represented thereby, and to increase its specific rotatory power from  $+204^\circ$  to  $+212^\circ$ , or even  $+214^\circ$ . From these data O'Sullivan came to the conclusion



that the pure dextrin would not reduce cupric oxyde; but in this opinion he is opposed by Musculus and von Mering, who say that the dextrin obtained from any amyllum under the influence of any starch-transforming ferment always reduces cupric solution, and that this reducing power can be destroyed neither by boiling the dextrin ten times with alcohol, nor by subjecting it repeatedly to the influence of yeast. These authors further admit that the reducing power of the substance obtained by the side of maltose varies between considerable limits, owing to the fact of the body not being always the same, but a mixture of several different bodies closely resembling each other, as of dextrins, similar in composition, but dissimilar in their power of reduction and rotation. The power of reduction, according to them, is the greater, the farther towards complete transformation into final products the zymolysis of the starch-molecule has proceeded.

According to O'Sullivan the zymolytic dextrin is a brittle white powder, without crystalline structure. It dissolves easily in water, and, if it has previously been dried at  $100^{\circ}$ , the solution is accompanied with a rise of temperature. In cold alcohol of sp. gr. 0.82 it is not perceptibly soluble, and alcohol which has been for three days in contact with the dry substance exerts no action on a ray of polarised light passed through a layer of it, 220 mm. in length. An aqueous solution containing in 100 c.c. 10 grms. dry substance, has a sp. gr. = 1.03845. Its specific rotatory power may be taken to be  $[\alpha] = +213^{\circ}$ . It is not coloured by iodine. Dried over sulphuric acid, its weight becomes constant when it contains 9.5 to 10 per cent. of water. This it completely loses in a current of dry air at  $100^{\circ}$ . The formula  $C_6H_{10}O_5 + H_2O$  corresponds to 10 per cent. of water. On elementary analysis it gives data corresponding closely with the theoretical 44.44 per cent. C, and 6.17 per cent. H, required by  $C_6H_{10}O_5$ .

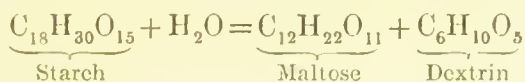
When a solution of this dextrin is treated with malt extract, its reducing power gradually increases, and becomes constant when the cuprous oxyde precipitated (weighed as cupric oxyde) is equal to 66 per cent. glucose, calculated on the dextrin employed. The specific rotatory power of the matter in solution is then  $[\alpha] = +150^{\circ}$ , having fallen from  $+213^{\circ}$ .



A solution of this dextrin can be boiled with cupric solution during from 12 to 14 minutes, without giving any reduction, but if the boiling (in presence of an excess of copper solution) is continued, cuprous oxyde is gradually formed, showing that the dextrin is changed into a reducing body by prolonged boiling with alkali. The action is so slow that any maltose or glucose, which may be present by the side of the dextrin, will have been destroyed some time before the dextrin commences to transform into the reducing body.

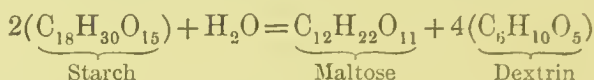
*Proportions between maltose and dextrin obtained under different conditions of zymolysis.*—Schwartz (Journ. Pract. Chem. 1, 1870, 212) observed that the action of the ferment, and the nature of the products of its action upon starch, depended to a large extent upon the temperature at which the fermentation was conducted. At temperatures varying from the lowest up to 60° he found an amount of reducing action produced corresponding, when considered as due to glucose, to from 50 to 53 per cent. of the liquefied starch. When the reaction was forced at a temperature of from 65° to 70°, an amount of reducing power was produced corresponding to only about 27 per cent. glucose. At temperatures varying between 60° and 65° he found amounts of reducing power corresponding to from 27 to 53 per cent. glucose.

O'Sullivan extended these observations, and made them much more accurate. He found that malt extract dissolves gelatinised starch at temperatures not below 10° and not above 63° almost completely, provided the gelatinisation was perfect. The solution which, if it was hot, must be cooled after from 5 to 10 minutes of action, and filtered, invariably contains maltose and dextrin, in proportions agreeing closely with 67·85 per cent. of the former, and 32·15 per cent. of the latter, the cupric oxyde reducing power being = 44·1, and the (specific) rotatory power  $[\alpha]_D = +170^{\circ}6$ . These numbers show that the reaction probably takes place according to the formula



O'Sullivan further found that when starch is dissolved by malt extract at any temperature between 64° and 70°, and the

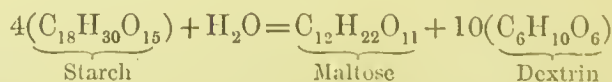
solution is immediately cooled and filtered, the product invariably contains maltose and dextrin in proportions agreeing closely with 34·54 per cent. of the former and 65·46 per cent. of the latter, the power of reducing cupric oxide being = 22·4 per cent. of glucose, and the (sp. ?) rot. power  $[a]_D = +191^\circ 8$ . These numbers make it probable that the reaction under these circumstances proceeds according to the formula



In this case, as in the preceding reaction, if the malt extract be not in excess the relation of the maltose to the dextrin is not materially altered by three or four hours' digestion at the temperature of decomposition; but if, on the contrary, the extract be in excess, or strongly acid, the dextrin yields maltose, and the maltose is partially converted into glucose. If the solution be boiled previous to digestion, the dextrin is not converted into maltose to any extent. If the unboiled solution be digested for a sufficient time with excess of malt extract, the greater part of the dextrin may be made to yield maltose, and by varying the time, and the quantity of extract, any proportion of dextrin and maltose may be obtained.

From these data it follows almost with necessity, either that dextrin has a much higher atomic weight than that assigned to it above, or that the maltose formed from it owes its origin to a synthetical reaction.

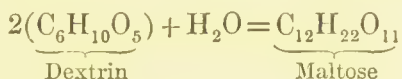
When starch is dissolved by malt extract at temperatures between from  $68^\circ$  to  $73^\circ$  and the point at which the activity of the transforming agent is destroyed, if the solution be cooled and filtered at the end of five to ten minutes, the product contains maltose and dextrin in proportions agreeing closely with 17·4 per cent. of the former and 82·6 per cent. of the latter, the (sp. ?) rot. power of the mixture being  $[a]_D = +202^\circ 8$  and the reducing power over cupric oxide = 11·3. These data are explicable by the following equation:—



A quantity of malt extract containing solid matter equal to

5 per cent. of the starch taken is in almost all cases sufficient to effect the transformation.

O'Sullivan is of opinion that the various decompositions expressed by the foregoing formulæ are due to some change brought about by heat in the character of the decomposing agent or agents. He expressly excludes, though by obscure language ('the various decompositions are not due to any difference in the size of the starch molecule'), the consideration of a molecule of starch containing more than 18 C, although the last reaction admits of the assumption of a molecule with 72 C, and all assumed minor molecules are obtainable by division of the largest one by 2 or by 4. He terms the transformation of starch into maltose and dextrin a *decomposition*, says that this decomposition is *molecular*, and does not take place according to one equation, but to three; he ultimately speaks of the conversion of dextrin into maltose as of a slow and gradual act of hydration. Of this he gives no formula, but he probably assumes it to take place according to the following one :



It is at once evident, that if the existence of this reaction be admitted, all maltose obtainable from starch may be assumed to be formed by the same reaction, as was practically maintained by most authors after Payen, and lastly again by Bondonneau (*Compt. rend.* 81, 1212). The consideration is not altered by their ignoring the existence of maltose, and speaking of the product as glucose. It is not difficult to explain that dextrin once formed, is less easily transformed into maltose, than gelatinised starch; inasmuch as most substances originating in a chemical reaction are more inclined to suffer or cause change in the nascent state, than after having assumed an independent free existence.

We may agree with O'Sullivan in the opinion that Bondonneau's dextrins were mixtures of the dextrin which colours iodine red, and the dextrin which does not colour iodine, with sugar under certain conditions, and torrefaction products; the objects having been extracted from commercial dextrin obtained by the action of heat on starch. But we must bear in mind

that O'Sullivan himself obtained by the action of malt extract on dextrin prepared by malt extract, a secondary dextrin, the specific rotation of which was only  $= +150^\circ$ , as against the  $+213^\circ$  of the dextrin from which it had been produced.

Musculus and Gruber obtained, by the action of diastas upon starch, three different kinds of dextrins which seemed to rotate the ray of polarised light the less the longer the diastas had acted upon the starch, and the larger was the amount of diastas which acted upon it or upon the primary dextrin separated from the maltose by fermentation or by alcohol. They all had a reducing power of 12, when glucose has a hundred, and differed only in rotation.

				After diastas (second)	
		Red. P.	Rot. P.	Red. P.	Rot. P.
N <sup>r</sup> . 1	. .	12	$+210^\circ$	36.5	$+159^\circ$
N <sup>r</sup> . 2	. .	12	$+199^\circ$	20	$+168^\circ$
N <sup>r</sup> . 3	. .	12	$+190^\circ$	12	$+190^\circ$

They gave no colour with iodine. With diastas the N<sup>r</sup>. 1 and 2 had their rotation power diminished, but their reducing power increased; N<sup>r</sup>. 3 remained in both respects unchanged; N<sup>r</sup>. 1 fermented strongly with yeast, N<sup>r</sup>. 2 slowly; N<sup>r</sup>. 3 did not ferment at all. From these data these authors conclude that there are at least two, perhaps three dextrins, which do not react with iodine, and are therefore termed (after Brücke, 'Proceedings of the Vienna Academy of Sc.' 65, part 2, p. 35 of the separate impression), achroodextrins, and distinguished from each other by prefixing the first three letters of the Greek alphabet, as  $\alpha$ ,  $\beta$ , and  $\gamma$  achroodextrin. The N<sup>r</sup>. 1 is  $\alpha$ -achroodextrin, the N<sup>r</sup>. 3 is  $\beta$ -achroodextrin; the N<sup>r</sup>. 2 they seem to admit to be a mixture. The  $\gamma$ -achroodextrin they obtain by allowing diastas to act upon starch in the presence of some alcohol during a whole year. This body had a rotating power of  $+150^\circ$  (the same as O'Sullivan's secondary dextrin obtained by the action of much malt extract upon primary dextrin) and a reducing power of 28. This dextrin does not ferment with yeast, but on being boiled with dilute sulphuric acid is transformed without residue into glucose. For this reason they propose that it should be termed end-dextrin. It can also be prepared by heating starch with dilute sulphuric acid containing 2 per cent. oil of vitriol,



until alcohol does not produce any longer any precipitate in the fluid. After removal of the acid by barytic carbonate, and destruction of the sugar by fermentation, the  $\gamma$ -achroodextrin alone remains in the solution. The same dextrin is obtained by the simultaneous action of diastas and yeast upon boiled starch; but in this case it is necessary that the amount of diastas be small, otherwise hardly any residuc is obtained.

The  $\alpha$ -achroodextrin is contained in beer brewed from strongly roasted malt, the  $\beta$ -achroodextrin in beer brewed from slightly roasted malt.

From so-called *soluble starch* (compare Musculus, *Compt. rend.* 1869, p. 1267, and *Ann. Chim.* 1874) these dextrins are distinguished by their solubility in cold water, in which soluble starch is insoluble; the latter acquires its right to the adjective soluble only at  $50^{\circ}$  to  $60^{\circ}$ ; its solution assumes a wine-red colour with iodine; in the dry state it is coloured blue by iodine; with an excess of this reagent it gradually becomes violet, yellow, and brown. Soluble starch has a sp. rot. power of  $[\alpha] = +218^{\circ}$ , and a reducing power of 6.

The *erythrodextrin* of Brücke becomes red with iodine, whether dry or in solution; it is soluble in cold water. Both 'soluble starch' and 'erythrodextrin' are easily changed by diastas. Neither substance seems to have been obtained in a state of purity.

The theory of Musculus and Gruber concerning the zymolysis of starch under the influence of starch-transforming ferments (including warm dilute sulphuric acid) is about the following. Starch has the formula  $n(\text{C}_{12}\text{H}_{20}\text{O}_{10})$ , in which  $n$  is not less than 5 or 6. The hypothesis of  $n = 6$  leads to the same number of atoms in the molecule of starch as O'Sullivan's last formula, namely to  $\text{C}_{72}\text{H}_{120}\text{O}_{60}$ . The effect of diastas or dilute acid upon this molecule is to split off a molecule of maltose leaving a dextrin ( $\alpha$ ) of the formula  $\text{C}_{60}\text{H}_{100}\text{O}_{50}$ , the one molecule of water taken up remaining with the maltose. The hydration and splitting off of maltose is now repeated, when ( $\beta$ ) dextrin,  $\text{C}_{48}\text{H}_{80}\text{O}_{40}$  is left; this by another loss of maltose leads to ( $\gamma$ ) dextrin  $\text{C}_{36}\text{H}_{60}\text{O}_{30}$ . This now is surmised to transform into maltose (threc molecules) directly, without any formation of dextrin corresponding to the terms with  $\text{C}_{24}$  or  $\text{C}_{12}$ .



It will be seen that O'Sullivan's data and theories offer great advantages over this hypothesis of Musculus and Gruber, which is very unsatisfactory when considered with relation to the quantities of products formed by different reactions and at different stages. These quantitative relations have been observed by O'Sullivan, and his results are therefore entitled to the greater consideration. But even so the question of the dextrin or dextrins formed by zymolytic agents is yet open, though that concerning the sugar or sugars formed by these agents is finally determined.

*Zymolysis of starch by saliva.*—The discovery by Leuchs, confirmed by Schwann (mentioned in the Introduction) that saliva had the power to transform starch into a kind of sugar, was generally formulated in physiological works to the effect that the sugar formed was dextroglucose. It is the merit of Seegen, and after him of O. Nasse (*Pflüger's Archiv*, 14, 1877, 473), to have shown that the sugar produced from starch by saliva is not glucose, but a sugar whose reducing power is nearly doubled by boiling with sulphuric acid. Seegen termed it ferment sugar, Nasse ptyalose; the latter also found that achroodextrin was formed at the same time, and that, as he thought, from impurity, it retained a certain reducing power. Musculus and v. Mering find that the ptyalose of Nasse is maltose, and that in fact the decomposition of starch under the influence of saliva (half a litre of mixed human saliva to a solution of 100 grms. of starch in 1200 c.c. of water digested during 6 hours at from 30° to 40°) is the same as that which it undergoes under the influence of diastas. About 70 per cent. of the starch employed are obtained as maltose; 1 per cent. passes into glucose by fission of a little maltose; the rest is obtained as dextrin, which always reduces copper solution; this dextrin is considered by them to answer to the description given of that substance by Musculus and Gruber in the paper above referred to.

*Zymolysis of starch by pancreatic ferment.*—The pancreatic ferment is employed as obtained in solution by extracting fresh minced pancreas with cold water. It is added to the hydrated starch, the mixture is heated for some hours to 40°, and then allowed to stand for some hours longer. The filtered liquid

is evaporated to a syrup, and this is treated with a large volume of alcohol, and to the filtered alcoholic solution ether is added. In this way dextrin remains insoluble in alcohol; the ether added at intervals produces several successive precipitates of maltose; the ultimate mother-liquor contains a little dextrose corresponding to about 2.6 per cent. of the starch employed.

*Zymolysis of hepatic glycogen by saliva and diastas.*—100 grms. of glycogen from the liver may be digested with 250 c.c. of saliva and 1 grm. of ptyalin or with 5 grms. diastas during some hours. By the processes above described Musculus and von Mering obtained from the digested fluid maltose and a little dextrose. The dextrin obtained from glycogen was different from that prepared from starch by the same ferments, for it formed a white powder, unchangeable by air, whereas the dextrin from starch appeared as a hygrophilic, easily dissolving mass. The dextrin from glycogen reduced cupric solution, and the power varied between 3 and 19, when the same weight of glucose reduces 100.

The amount of total reducing power which is developed in solutions of starch and glycogen under the influence of diastas, saliva, and pancreatic ferment varies between wide limits: it is after some hours, on an average, about 50, but increases on standing for days and weeks to 65. Glycogen is less energetically transformed by diastas than by saliva. Glycogen with saliva yields not rarely only a reducing power of from 34 to 41, while with pancreatin it yields a reducing power of from 45 to 48 per cent. of its weight of dextrose. (Compare Seegen, *Centralbl. Med. Wiss.* 1876, N. 48, also Nasse, *loc. cit.*)

The liver after death contains, according to Nasse, a sugar, the reducing power of which cannot be increased by boiling with dilute sulphuric acid: consequently dextrose. This is confirmed by Seegen (*Archiv. Physiol.* 19, 1879, 123). This author pressed about a kilo of liver substance, subjected the pressed out juice to dialysis, evaporated the dialysate, treated the residue with absolute alcohol, and added to the solution freshly prepared solution of caustic potash in alcohol. The sugar-potash was deposited as a delicate yellow varnish-like mass. This was washed with alcohol, dried over sulphuric acid, dissolved in a little water, and tested by the polariscope, the

copper reduction, and the fermentation tests. It was dextrose-potash, as formerly described by Seegen (*Sitzb. Acad. Wiss. Wien*, vol. 64). Musculus and von Mering had also found dextrose in the liver, though they add that maltose was also certainly present. The presence of dextrin in the liver they were unable to prove with certainty.

The opinion formerly entertained by Seegen (*Diabetes Mellitus*, 1870) and Schtscherbakoff (*Ber. Deutsch. Chem. Ges.* 1870) that there might be two different kinds of glycogen produced, the one after amylaceous, the other after albuminous diet—an hypothesis already controverted by von Mering (*Arch. Physiol.* 14, 1877, 283), has been finally abandoned by Seegen.

The transformation of starch and glycogen into glucose by whatever intermediate stages is accompanied with the loss of the colloid, and the assumption of the crystalloid state. The change is therefore away from the organoplastic direction, and towards the formation of products which are unfit for the formation of organs, but fit for the production of power by oxydation.

## IV.

*THE LIFE AND PHILOSOPHY OF ROBERT JULIUS  
MAYER, PHYSICIAN TO THE TOWN OF HEIL-  
BRONN.<sup>1</sup>*

ROBERT JULIUS MAYER was born November 25, 1814, and died March 20, 1878. His father was a pharmaceutical chemist and owned the establishment known as 'Apotheke zur Rose' at Heilbronn on the Neckar in Würtemberg. He was a man of high attainments and entirely devoted to his profession and the sciences collateral to it. He was rarely seen in company, but spent nearly all his free hours in the large rooms of his house which were filled with chemical and physical apparatus, botanical and mineralogical collections, books and pictures; he was a short, stout man with a large head and large eyes. His wife, the mother of the object of this notice, was a busy housewife of great goodness, but no other prominent qualities, except a faculty which she shared with her husband of being easily excited to great eruptions of anger by comparatively trivial circumstances capable of producing displeasure. This latter peculiarity was inherited by their three sons, more particularly by the eldest and youngest.

The eldest brother, Fritz by name, was about eight years older than his youngest brother Robert: he was talented, of high character, and full of knowledge; he was even better in-

<sup>1</sup> We take from a description of his life by his friend G. Rümelin entitled 'Erinnerungen an Robert Mayer' in the *Augsburger Allgem. Zeitung* of April 30, 1878, the data concerning the course of his life, his development, and his mode of inquiry, but regarding his philosophy we shall be guided by a necrologue in the *Schwäbische Chronik* of April 6, and by Mayer's collected works, second edition, published at Stuttgart in 1874 under the title: *Die Mechanik der Wärme*.

formed than the father, more animated and given to conversation, and devoted to the cultivation of natural science; he is supposed to have exercised much influence upon the youngest brother Robert. Robert therefore grew up in an atmosphere of scientific views, occupations and conversations, and developed in it his inborn talent with the eagerness springing from interest. As a boy he was familiar with the use of the air pump, of several varieties of electric apparatus, with elementary chemical experiments and processes of production; he could diagnose most plants according to the system of Linné, and knew the contents of all current books used in the pharmacy of his father; from his brother Fritz he had acquired a knowledge of algebra and the use of logarithms, but his instruction in these branches of knowledge was not methodical, and no great assiduity was given to it; this explains much that remained imperfect in the performances of his productive years.

Robert was an indifferent pupil at school. His otherwise excellent memory could not be exercised upon all ordinary subjects of instruction, but it could only be used for those in which he took an interest. To these latter the ancient languages did not belong. Now as in the gymnasium success mainly depended upon proficiency in the classical languages, and as his proficiency in other sciences had no opportunities of showing itself, or if it did, was not observed, he was looked upon as a mediocrity. The boy was physically neither strong nor dexterous, but he had an extraordinary amount of perseverance and staying quality, particularly in walking; this he cultivated, and when a student he on one occasion made the way from Tübingen to Heilbronn, a distance of 77 kilometres, in between 14 and 15 hours.

Robert had a great liking for all kinds of games, and acquired great proficiency at chess, several varieties of games at cards, billiards, and ninepins; it is related that he carried all rules to excess, but played more for the enjoyment of the theory of the game than for the sake of winning.

Rümelin was intended for the church, and in 1828 left Heilbronn to enter the theological seminary at Schöndal, a former monastery some twenty English miles to the north-east of Heilbronn upon the river Jaxt. Robert severely felt the loss



of his friend, and prevailed upon his father to send him as a private boarder to one of the professors of the seminary; there he participated in the instruction given in the seminary, but as the foundation scholars had all been selected by competition from a number of applicants three times as great as the number which could be received, they were well instructed and Mayer's deficiencies in classics were the more apparent, and his place was always amongst the lowest in his form. But it began now to be felt that he had to be measured with a different measure, as he knew many things of which the seminarists had no idea, and he began to be esteemed by his schoolfellows as well as by the masters; for he was always open-hearted and truthful; all he said bore the character of originality; his mode of expression became often aphoristic; humour and poetic quotation gave to his conversation a rare attraction, and when he grew lively most boys looked at him with expectation; to some the fireworks of his changing ideas were not agreeable.

The professor with whom Mayer boarded was Wilhelm Klaiber, who had married a sister of Wilhelm Hauff the novelist. To this lady he was much attached, and grateful for admonitions which from others he did not so easily tolerate.

Some entertainments with the aid of the magic lantern to which he here treated his schoolfellows, at this period obtained him a sobriquet, which he retained through life: he had caused 'spirits' to appear on the wall, and accompanied their apparition with recitations and improvisations of his own; from this his schoolfellows termed him 'der Geist' which literally means 'the spirit,' but has the additional significance, no doubt intended to apply sarcastically to Mayer, of 'the genius'; this had the effect of singling him out for ever from the great number of Mayers, which in Germany are as difficult to distinguish from each other as in England are the Smiths, Browns, and Robinsons.

In this seminary Mayer spent three years, and as he learnt only a moderate amount of classics, but was not at all advanced in the branches of science for which he had talent, it may be said that these three years were entirely lost to him. In 1832 he passed the examination called of maturity, corresponding to the matriculation examination of English universities, and then entered at the age of  $17\frac{1}{2}$  years the university of Tübingen to

study medicine. He did not attract the attention of his teachers, amongst whom were Autenrieth and the two brothers Gmelin (Ferdinand and Christian), by any prominent quality. He attended the purely medical lectures, no philosophical ones, none in history or philology, although Strauss and Vischer then were great attractions; physics he attended, but at a time when the course was given by a private teacher (privat-docent) who only filled up the vacancy until a new professor should be appointed. Nörrenberg, well known by his researches on polarised light, was appointed, but Mayer did not attend his lectures. On the whole he paid attention only to medical studies, and heard the lectures on systematic anatomy several times over. The higher mathematics Mayer acquired only when he was already a practising physician at Heilbronn, from Professor Bauer, now at the polytechnic school at Stuttgart. On the whole, although Mayer followed the medical lectures with interest and regularity, he did not study in his free hours and burnt no midnight oil except for cards and social parties. He made many friends whose attachment he preserved through life; of these several like Wunderlich and Griesinger passed away before him.

In his later university years he was induced to become a member and office bearer of one of the peculiar societies of students termed 'corps'; he fought some duels, learned riding on horseback, and the art of singing, though in this latter he had shown no aptitude. But all this did violence to his entire character, which was not made for either representing or directing. As the Frankfort Diet prepared to suppress the society, like so many others all over Germany, the society or corps determined to dissolve spontaneously, and in 1836 carried out and announced this resolution.

But notwithstanding this spontaneous action, and because some social cohesion continued to exist between the former associates, the university authorities instituted proceedings against them, and as the result of these the founders and office bearers of the society, amongst them Griesinger and Mayer, were compelled to leave the university for some time (*consilium abeundi*). During the investigation Mayer like others was in confinement (*carcer*), and it was here that the first

traces of that disposition to mental aberration showed themselves, which destroyed the happiness and usefulness of the later years of his life. He refused all food, and drank water only; the physician who examined him upon the report of the pedell found him without objective symptoms, but upon Mayer's special demand, bled him twice in the arm, to remove congestion, of which he complained; on the sixth day of confinement the physician reported to the university judge that 'Mayer could not be considered as completely deranged, but that he was in a state which could easily pass into madness.' Upon this report his confinement was altered to arrest upon parole at his own residence. The 'advice to leave the university' in his case was given on the ground above stated, coupled however with the collateral reasons that he had been at a ball of the casino in a frock-coat instead of the obligatory dress-coat. This latter imputation, which was actually untrue, casts a vivid light upon the proceedings resulting from the participation in the society. However, the consilium in Mayer's case was of no practical consequence, as he had completed his course of studies of five years, and therefore left Tübingen as a matter of course.

During the summer and autumn of 1837 he visited the hospitals of Munich and Vienna, and attended the clinical lectures delivered in them. In January 1838 he was admitted on petition to present himself for the first medical examination at Tübingen, and passed it; in the summer of the same year he also passed the major examination at Stnttgardt, in the second class, first division. In chemistry he gained honours. His examination papers were described by the examiners as 'showing thorough knowledge and independent judgment.'

He returned to Heilbronn and began the trials of a young doctor. But his impatient desire for activity and a sight of distant lands caused him to aspire to an appointment in the medical service of the Dutch Colonial office. With a view to this he studied the Dutch language. He also acquired some knowledge of French, and went to Paris. In the latter town he lived for some time together with Griesinger and Wunderlich. Thence he went to Holland, passed the examination, and received the diploma as 'Officer of health.' Impatient as he

was, he accepted the first post which offered itself, and sailed for Java as surgeon on board a merchant-ship.

The voyage was accomplished without any incident worthy of note ; but during it was developed to the state of consciousness that train of ideas by which he has made his name known to philosophers, and ranged himself as one of the foremost amongst them.

In him there lived the desire to know things through his own contemplation, and find their connection by meditation ; to make out the causes of phenomena, and in search of them to travel to the most distant regions of that which could be recognised by the understanding ; he had, says his friend and biographer Rümelin, the fateful gift to live always entirely for and in one subject, and to press the whole store of his ideas into the service of that subject ; he was purely truthful without presumption or prejudice.

Mayer had provided himself well with medical works and surgical instruments, and by the side of them he carried meteorological, physical, and astronomical apparatus. To the study and use of these almost his entire time was devoted, for during the eight months of the actual sea-voyage, not a single person on board became ill, and his conversation was confined to moderate intercourse with a few officers, the low-Dutch of the common sailors being unintelligible to him. He was therefore practically in solitude, and reduced for entertainment to the resources of his own spirit. He had never been a quick or continuous reader, particularly because what interested him always reduced him again to play with his own ideas. But he could for long periods fix his entire attention, his eyes, and his mind, upon any feature of nature, upon clouds, wind, or water ; he loved to watch a thunderstorm through all its phases from beginning to end. This inclination he could now satisfy as never before upon the grandest subjects : upon the ocean with the phenomena of flood and ebb tide, the passate-winds (trades), the calms and storms ; upon the starry sky of both hemispheres, particularly the southern one with the new signs ; upon the results of the powerful action of the tropical sun ; upon the organic life of the eastern islands, particularly Java ; upon a new vegetable world ; upon a series of great volcanoes, partly extinct,



partly in action. All this he saw, not with the eyes of the painter or poet, but with those of the meditative inquirer, who wants to get a glimpse of the kind and cohesion of the most elementary powers of nature.

Mayer relates that relatively small incidents directed his thoughts into particular channels. Thus the pilot had one day told him, that the waves which had been much agitated by a storm were sensibly warmer than the waters of the same sea in a state of quietness. When at Java he had to bleed some of the sailors, and was surprised to find that the venous blood was of a light colour, much resembling, as he believed, arterial blood. When he mentioned this observation to some of the local physicians with whom he came in contact, they confirmed his observation, and informed him that the phenomenon was observed upon natives as well as Europeans. The Dutch doctors, however, warned him against bleeding his patients, as the practice was, in tropical climates, condemned by experience. Mayer then began to consider how the phenomenon could be explained, and recognised at once that the explanation offered, namely, that the great redness of the venous blood was an effect of the greater heat of the sun, was in fact no explanation at all. This led him to consider the relation between mechanical labour and organic heat, and the wants of the body as regards food. Men had been satisfied with the enunciation that friction produced heat, but Mayer was not satisfied with it. He searched for a law of causation of these phenomena, which he felt darkly were in connection with each other. And this search now occupied his mind entirely. The single steps which led him to his goal, the links of the chain of his thoughts, the history of his gestation so to say, has not been preserved by anything which he either wrote or said. But he stated repeatedly that it had been necessary for him to overwhelm various errors and half-truths, and that he had been obliged to make many artificial concessions to existing doctrines, before he could formulate his early conceptions as a presentable hypothesis. It is however certain that he brought the fundamental idea of his subsequent writings home with himself from this sea-voyage, and that the origin of this idea was in his genius and in a ceaseless penetrating energy of thought. That motion could be transformed into heat, heat



into motion, in proportions capable of being ascertained by experiment, was one of his earliest convictions; then came the further prospect: not only matter is indestructible, but force also; the forces of nature do not perish during or in their action and effect, but they are only changed, and continue to exist in the changed form.

In February of the year 1841 Mayer returned to his native town, mainly occupied with the wish to elaborate his idea into all its details, to provide it with the support to be derived from literature and experiment, and to submit it to the learned. He knew that the principal point which he had to prove, before his idea could be called a discovery, was the finding and ascertaining the so-called equivalents of the forces which he considered to be related to each other. For it would be of no use merely to assert in general that heat and motion were mutually convertible into each other, unless it could be stated and proved that a constant relation obtained, that to a certain measure of heat always corresponded a certain measure of motion. He now began a series of experiments, the results of deduction clearly; these he has himself described in his works; they led him at first to the result that by the fall of a body of a certain weight from a height of about 365 metres an amount of force was produced, which by the sudden arrest of the body, would be let loose in the shape of heat, and that the amount of heat so produced was capable of raising the temperature of a weight of water equal to that of the falling body, from  $0^{\circ}$  to  $1^{\circ}$ . He subsequently found this relation still incorrect, and the height of fall required to be greater. But this detail was insignificant compared with the enunciation of the equivalents of heat and motion as an existing law.

Open-minded and colloquious as Mayer was, he set about to make converts for his new doctrine. He spoke to his colleagues of the medical profession, to the teachers of the higher schools, and in the summer of 1841 made a journey to Stuttgart, Tübingen, and Heidelberg, in order to communicate his ideas to philosophers of note, or to any who would listen to them, and hear their opinions. But these attempts were not encouraging. The matter being new, and Mayer's manner of speaking being not of the kind which quietly develops, but striking and aphoristic,

and contrasting strangely with the prevalent diction of hand-books, he was looked upon with distrust; the young man of 27 years of age, who though known to his friends as imbued with original ideas, was unknown in the world of learning, and wanted at once to change the entire aspect of the science of physics, was listened to with impatience as a hawker of paradoxes; he got for answers shrugs of the shoulders and shakings of the head, or he was asked whether he had read certain chapters of certain books; and when he had to reply in the negative, the conversation ended with the recommendation to do so, and then to again examine his ideas. In later years he excused his early interlocutors with the admission that he himself had then not been quite clear on all the bearings of his theory, and that he had not yet recognised the necessity of rejecting the old doctrines, which his theory superseded, entirely, but had been entangled in the belief that they could be reconciled by artificial hypotheses. One man however received him well, listened to him with an open mind, and encouraged and aided him; this was Professor Jolly of Heidelberg; and of him Mayer has in the note given to his biographer spoken in the highest terms of grateful recognition.

Mayer never read a so-called philosophical work; Rümelin once gave him Hegel's *Logic*, and the volume of the *Encyclopædia* which contained the so-called '*Naturphilosophie*,' but Mayer returned them after a few days, with the statement, that he had not comprehended anything of what he had read in them, and that he could not understand these works were he to read them for a hundred years. He had at that time some axiom-like aphorisms, which he repeated frequently, uttering them to his friends on meeting and on leaving them, sometimes shouting them after them, when they had just left his presence. *Ex nihilo nihil fit; nihil fit ad nihilum. Causa æquat effectum.* He challenged all comers to state what could be said against these propositions. Rümelin has preserved a note of an argument which he had with Mayer concerning these theses; he had objected that indestructibility was not an attribute of the phenomena of organic and psychic life, and the thesis *Nihil fit ad nihilum* was not generally valid. In consequence of this Mayer excluded *ad interim* organic and psychic phenomena from his hypothesis, and confined it to the physical phenomena,

those termed in physics 'forces.' He took however on one occasion an illustration from the transformation of force which takes place when four horses draw a stage-coach from one place to another, say from Heilbronn to Oehringen (he was walking with Rümelin on this road and the stage-coach passed by during the discussion), and his then interlocutor ingenuously states that from this illustration he learned for the first time what were Mayer's real intimate thoughts and philosophic objects.

Towards the end of 1841 Mayer wrote the small paper, modestly entitled 'Remarks on the Powers of Inorganic Nature.' It may be supposed that the apposition of the adjective 'inorganic' was the result of his conversation with Rümelin, above alluded to; this adjective, he in later years judged to be not quite appropriate; it was certainly unnecessary, as the rest of the title was a sufficient limitation of his purpose. He forwarded the manuscript to Poggendorff for insertion in the *Annalen der Physik und Chemie*, which certainly would have been the proper medium for the publication of the essay. But Poggendorff declined it as unsuitable, and returned the manuscript to the author. The latter now sent it to Liebig for insertion in the *Annalen der Chemie und Pharmacie*, and here it was accepted. How great was the satisfaction with which Mayer received this information is shown by a sentence in his short biographical notes, which also introduces the reader to a new development in his life. 'In the month of May (1842), in the same hour in which I introduced to my aged parents my affianced bride, who was to found the happiness of my domestic life, and who has since stood by my side as my faithful wife, I received a letter from Giessen in Liebig's handwriting, in which I was informed that my first essay on the mechanical theory of heat had been accepted for insertion in the *Annalen der Chemie und Pharmacie*.'

The name of the bride was Wilhelmine Closz, the daughter of a respected and wealthy merchant of Winnenden. Another daughter of the same house being engaged to a brother of Rümelin, the biographer, from whom we quote, it was desired that both marriages should be solemnized simultaneously, which was accordingly done, Rümelin, who was in holy orders, officiating on the occasion (August 14, 1842).

Mayer now entered into that period of his life which, though short, was the most productive of philosophical work of value to the world, and heartfelt unclouded happiness to himself. His scientific publication received neither notice nor recognition, but he had a rapid success in medical practice, which made his circumstances, already easy and almost independent of professional exertions, affluent and secure, at an age at which others have to sustain their hardest struggle. Young as he was, he received the appointment of county surgeon (*Oberamtswundarzt*), and soon was able to exchange it for that of physician to the town of Heilbronn and to the poor of the town (*Stadt- und Städtischer Armenarzt*). In his character of physician he was cautious, circumspect, and observant: heroics were not in his list of remedies; the experiments which he had in younger years sometimes made upon himself, he never attempted upon his patients. Medicine he said, is not a science, but an art, the *ars medendi*. Every case, he averred, had to be considered by itself, and had to be treated according to the rules of an eclectic empiricism, in which the experience *ex nocentibus et juvantibus* must be the guiding principle. He was fond of quoting a saying of his teacher Autenrieth, that every medical system stood in the same relation to nature as the tangent stood to the circle: that it touched only in one point, and (considered as a progressing phenomenon, we suppose) would again go to a distance from it, unless it was modified and by a new breaking force turned again towards the periphery. (Vain endeavour of the writer to make a tropus logical in its further progress.)

The biographer continues by stating that Mayer was not entirely absorbed by medical practice, but that the practice went alongside of the continuation of his inquiries in natural science, and had an intimate connection with them. The essential result of his mental activity during these years was the final elaboration of the fundamental idea of the indestructibility of the forces, and of the relation between motion and heat, the final exclusion of the residues of former scientific theories which were supplanted by his own, and the following out of the results of the application of his theory to all approachable problems. The relation of motion to heat now became only an instance, an



example of a general law of universal dominance; 'there are not many forces which accidentally exist and act by the side of each other, but there is only one living force, which circulates eternally through changes of form.' Physics became the teaching of the metamorphoses of the forces. Astronomy was no less fructified; chemistry, physiology, and pathology participated in the impetus which the recognition of this law gave to all philosophical studies. Mayer now wrote the two works upon which reposes his claim to the scientific distinction which is now accorded to him by philosophers all over the world. The one published in 1845 bore the title,<sup>1</sup> *Organic Motion in its Relation to the Change of Matter (in the Living Body)*. A *Contribution to Natural Science*. In this case the title did not cover the contents. The second work, published in 1848, was entitled <sup>2</sup> *Contributions to the Dynamics of Heaven*. Both essays are now considered as classical works in German natural science, not only on account of the novelty of the subject and the amount of new information which they offered at the time, but also on account of the unquestionably masterly mode, and the lucid and terse style in which the subject was presented. These works are now translated into many languages; but at first Mayer could hardly find a publisher, and of the first work he had himself to pay the expenses of printing and publishing.

The year 1848 disturbed this period of happiness and productiveness. Participating for a short time in the enthusiasm of the so-called 'Days of March,' Mayer soon placed himself decidedly on the side of authority, and became a conservative in word and action. His brother Fritz, on the contrary, became a leader of the most advanced radicals; and as both were rigid in their convictions and carried them into effect, they were soon opposed to each other as extreme types of opposed factions. In 1849 Fritz Mayer became a leader of insurgents who went in aid of the Baden insurgents. The wife of Fritz requested Robert to exercise his influence to cause her husband to return, and Robert travelled after his brother. But he was recognised, arrested, and ran great peril of being shot as a spy or enemy of

<sup>1</sup> 'Die organische Bewegung in ihrem Zusammenhang mit dem Stoffwechsel. Ein Beitrag zur Naturkunde.'

<sup>2</sup> 'Beiträge zur Dynamik des Himmels.'



the radical cause. The leader Sigel however set him at liberty at once, and he returned to Heilbronn. He had not shown either fear or feebleness, but the commotion of his mind had been extreme, particularly on finding that his second younger brother had also become an insurgent leader. About the same time, namely, in spring 1849, he was deeply afflicted by the death of two of his children.

Mayer had lived in the hope that his scientific publications would find, if not assent, yet some attention and recognition amongst physicists. But seven years had elapsed since the publication of his first paper, four years since that of his first work, and a year and a half since that of his third, without his having received a single friendly word of encouragement from anyone. Attacks however and derogating judgments there had been, but the bulk of the learned in physics took no notice whatever of his philosophy. Some disputed the priority of his discovery, others its value. The English physicist Joule had, quite independently of Mayer, and about a year later, found the equivalents of heat and motion, by a method of his own, and determined the distance through which a body must fall in order to produce the unit of heat, more correctly. He had however treated the question as a special problem in physics, and not drawn the general conclusions as to the indestructibility of the forces, and the other important results attached thereto. This led to a dispute regarding the priority of the discovery, which was subsequently decided in favour of Mayer, without prejudice to the independence of Joule. Mayer put forth his claim to priority in a letter to the French Academy of Sciences, and the *Comptes rendus*, 1845, reported on the relative claims of Mayer and Joule very impartially.

Four years after these discussions, and when Mayer's mind had suffered much from the events which we have described above, he was induced by the desire to secure priority, to publish in the extra sheet of the *Allgemeine Zeitung* of May 14, 1849, a short notice, entitled 'Important Physical Discovery,' with his name attached, in which he announced that he had succeeded in finding a simple means to show experimentally the transformation of motion into heat, described the apparatus he had constructed to that end, and claimed the priority of the

discovery of the principle of the transformation against an article in the *Journal des Débats* which had disputed it.

A week later there appeared in the extra sheet of the same Journal of May 21, an article by Dr. Otto Scyffer, then a privat-docent of physics at Tübingen, in which Mayer's publications were spoken of in an almost contemptuous manner. Among other things the writer said, that for the professional man no discussion of Mayer's work was requisite, but laymen might find an explanation 'according to the position of science' desirable. He continued by denouncing Mayer's article in the *Annalen der Chemie* as a mass of untenable views concerning the forces of nature. The confusion which reigned in this article between the ideas of force, cause, effect, etc., and the deductions therefrom had been sufficiently exposed in scientific journals. Mayer's theory, the writer continued, was a completely unscientific paradox and in contradiction to all clear views on the action of nature. To the apparatus described by Mayer was denied the character of novelty, and it was added that it did not prove what it was intended to prove. In this tone the entire article was conceived; it sounded more like the correction of a fool by an indignant master, than the efflux of fair controversy.

Mayer, who was not in the least aggressive, but got into great excitement when any attack was made upon him, felt quite heartbroken by this as he termed it 'public insult in so respected a newspaper.' To a reply, which he sent to the editor, admission was refused, and all further steps to obtain redress remained without result.

Finding himself thus treated with rancour and contempt, without a word of comfort or approbation from any of those to whom his naturally affable mind looked for sympathy, unable by the habit and nature of his mind to liberate himself even for a time from oppressive thoughts by diversions such as constitute so common a resource of most mortals, he became a prey to wounded pride and unsatisfied ambition. All arguments and entreaties of his family and friends were in vain, the excitement rose to a real frenzy, an inflammation of the brain; this having already abated, he was, as he wrote himself, in the early morning of May 28, 1830, the heat of the season being unusually great, and he having partly in consequence of the heat spent a

sleepless night, seized by a new access of delirium, during which he jumped out of the window on the pavement nine metres below the window. He alighted on his legs, which were greatly injured, though not broken. During the days following this misfortune he battled with death, but gradually improved, and after a long and painful sickness he could resume his ordinary avocations and his practice. The right leg remained weak during the rest of his life; he had to drag it after him, and to walk with the aid of a stick. After this outburst he became more quiet, and, comforting himself with religious sentiments, based on the hypothesis of earthly punishment for moral faults, returned to his labours, and wrote *Remarks on the Mechanical Equivalent of Heat*,<sup>1</sup> 1850. This added nothing essential to his former publications, and was claimed in fact to be only an appendix and supplement to them. But it is important as showing that his power of writing was unimpaired; nobody could discover in it evidence of a deranged mind.

However Mayer had now entered upon the dark period of his life; he lived yet 28 years after the first attack of mania, and these years, says Rümelin, must be considered as principally occupied by repeated outbursts of mental derangement. These had the character of mania, without delusions or fixed ideas, and made it necessary, in view of what had occurred, that he should be removed to an asylum; when he was somewhat more quiet he went to the asylum of his own free will. It appears that he was unsuitably treated at the lunatic asylum at Winnenden, and during the remainder of his life expressed himself as greatly offended by what he had there experienced. In later years he visited the asylum at Kennenburg, where he was treated as a volunteer and guest, and not subjected to any restraint whatever. A few weeks of residence in this place had the effect of making him quiet for longer periods, during which he could return to his family.

In such quiet periods he wrote yet several essays, such as the lecture which he gave in 1869 at the Scientific Congress at Innsbruck, 'On necessary Consequences and Inconsequences of Heat-mechanics.' 'On the Significance of Unchangeable

<sup>1</sup> 'Bemerkungen über das mechanische Aequivalent der Wärme.'

Magnitudes;' 'On Nutrition;' 'On Fever;' 'On Earthquakes;' 'On the Empty Space of Toricelli;' 'On the full Exchange of Forces.' (The German word for this latter idea is *Auslösung*, and means literally the redeeming of a pledge; the expression is now very frequently used, particularly in experimental physiology, and not always appropriately). He invented a dynamometer, for which the Board of Trade of Würtemberg caused a golden medal to be given to him. Thus we see that his powerful mind was yet active and clear in the intervals from the greatest of misfortunes which can befall a human being.

Having always been opposed to materialistic views he now turned decidedly towards a positive religious belief; it formed itself into, what Rümelin terms, a subjective theology, meaning a theology of Mayer's own invention; this at one time took the shape of a proposition for the fusion of the Roman and Protestant Churches. His ideas became freaks (*Schrullen*) which his friends discussed with leniency, and which did no harm. Politically he always had been an adherent of the principle of authority, of what may be termed extreme conservatism. This in 1866 made him sympathise with Austria, but in 1870 the victories conquered him too, and after the battle of Wörth, he came to his friend Bishop Lang, and said to him the words of Job, cap. 42, 3: 'I confess that I have spoken unwisely.' After that he was a good imperial German.

In these later years he also experienced much pleasure and satisfaction by seeing his work recognised; by feeling the glory which was to surround the recollection of his name; by finding himself honoured as the first philosopher of his time; by finding it said and admitted that his discovery had effected an immense progress in the natural sciences, and had opened new fields of thought and action.

Schönbein was the first to recognise the importance of his writings, to visit him and befriend him; and the first public manifestation of recognition which he received was the diploma of corresponding member of the Natural Science Association at Basle (1858). After this, attention was directed to him by Liebig, in an oration delivered at Munich on a festive occasion, and then a wide circle of savants began to take notice of his researches. The university of Tübingen, his *alma mater*, where



he had taken his degree of doctor of medicine, also nominated him *honoris causa* doctor of philosophy and natural sciences. Diplomas arrived from Munich, Vienna, Turin, Halle, Frankfort, and from other learned societies too numerous to mention. The French Academy appointed him a corresponding member, and awarded him the prize Poncelet. The Royal Society gave him the Copley medal. In England he was mainly introduced to the notice of scientific circles by Tyndall, in France by Verdet, in Italy by Count St. Robert. Tyndall established closer friendly relations with Mayer, and it was for Tyndall and at his request that Mayer wrote a short autobiography, from the draft of which Rümelin has quoted and we have repeated above some passages and data. In 1867 Mayer received the order of the Crown, and with it the title of personal nobility.

These late successes helped to give to the evening of his life a happier aspect. But on the whole, says Rümelin, Mayer must be added to the number of those men who pay for the benefits which they render to mankind with the happiness of their lives. No doubt the principal reason for his misfortune was an unfavourable disposition of the brain and mind, a very great excitability, and long endurance in the excited state; inability to divert his thoughts from an indifferent object, from an adverse impression, or from an injury received; and a rigid inflexibility of his will. But Rümelin points out that it was not to the credit of German science—which was possessed of an immense apparatus for the attainment of its objects, numerous general and special journals, in which the most insignificant performances yet find room, and praise by accident or agreement—that a scientific discovery of the first order, one opening a new era in philosophy, should be liable to be kept out of sight by premeditated silence, or to be treated with contempt. This is mainly caused by the cohesion of cliques, and their trade protective principles; and in this enlightened age there still happens what is commonly imputed to the dark ages, that the innovations of genius are referred to the gratitude of later generations.

Mayer's features in younger, happier years had been florid, serene, and friendly; in later years they bore the expression of grave or sullen seriousness. In stature he was of medium height,



and well made, but his carriage was negligent and leaning forward; when walking he stared before himself on the ground, betraying immediately his meditating preoccupation. His senses were refined and attentive, but he had no disposition for art.

In later years the attacks of excitement became less frequent, and were very short. His children developed and gave him satisfaction. But his health began to fail, and in January, 1878, he was attacked with a serious affection of the lungs. To this he succumbed on the evening of March 20, when a quiet death, as his friend says, 'brought to this storm-tossed heart the desired rest.'

He was buried on the birthday festival of the German Emperor, a great concourse of people following him to the grave; the town of Heilbronn was in mourning, and the flags, so plentifully exhibited in honour of the day, were by request of the authorities removed during the hour of the funeral; his friend G. Rümelin spoke a funeral oration as delegate of the university of Tübingen, and could truly describe him as one of the most honoured masters of German science.

Mayer's mode of working was in the main *deductive*, and only to a small extent experimental. In this respect he was Aristotelic almost in the extreme. This limitation in one sense protected him no doubt from a fault, which has been committed by, or perhaps not rarely been imposed upon, experimental inquirers, namely that of being drawn into laborious and costly details by the want of, or desire for, more and better scientific data. He had a large body of generally admitted scientific data at his disposal; they were no doubt a disconnected, unorganised mass, but they were truths, and for these he found the connections by thought. When he had well observed a phenomenon he considered it as a riddle to be solved, and his energetic and passionate, abiding nature left him no rest until he had 'scientifically penetrated the phenomenon, and found the principle, of which the process was the simple effect.' He started with a deep conviction, one without alternative, a kind of instinct, that there was complete harmony between the laws of thought and the objective world, and to prove this harmony by the results of experience he conceived and declared to be the

most interesting and the most comprehensive problem which can be imagined.

That motion was the result of power, that this power could be shifted without transformation from body to body, was clear; it followed that motion was a form of power. But motion disappears under varying conditions, and apparently without any effect; but in many cases on closer observation an effect of disappearing motion is perceived: that effect is heat; on further observation it is perceived that heat is the only effect of disappearing motion, that there is no other effect but heat to be made out. Upon this datum of experience Mayer formulated the rejection of the teaching of the branch of science called physics, which involved the assumption that there could be a cause without an effect, and an effect without a cause, and substituted the hypothesis that heat arises from motion, that heat is transformed motion, that as motion is transferable power, heat is power, heat is force, and as originating from motion is transformed force. But in order to make of this hypothesis a scientific truth, there was wanted a numerical expression for a quantitative estimate (quantation) of the amount of heat produced by the transformation of a given amount of motion. Of motion the best known example was that which results from gravity, from the falling of bodies, previously lifted, towards the point of attraction. Mayer therefore set about to ascertain what amount of heat was produced by the fall of a body of given weight through a given distance, or taking the amount of heat as the unit to which the other data of the experience were to be formulated, he expressed the problem thus: To find the distance through which a body must fall, in order to be able to produce by the transformation of its falling power an amount of heat which shall be capable of raising the temperature of a weight of water equal to the weight of the falling body from zero to one degree of the Centigrade thermometer.

Mayer approached the solution of this problem by estimating the distance through which the body must fall as 365 metres. It was subsequently found and recognised by Mayer himself that this number is too small, and that 425 metres, as ascertained by the experiments of Joule, and confirmed by those of Regnault concerning the heat and relations of pressure of

gases, which have to be taken into account in the experience, is the more correct number. But this is a mere detail, an error which detracts nothing from the achievement of his relating a mechanical act, fall, to what was believed an independent potential effect or even a matter, to heat namely, and showed that they stand in proportions to each other which can be numerically expressed; that he established the existence of *the law of the mechanical equivalent of heat*. This result was not obtained with the aid of the higher form of mathematical operations termed analysis (a knowledge of this branch of science Mayer acquired only in later years), but with the simplest means of mind and matter. The one aid was the sharp discrimination of his understanding. Since the time of Newton it had been taught in philosophy that gravity was the cause which kept the universe together. Mayer recognised that in the word 'gravity,' as well as in the word 'force,' there was implied an inadmissible confusion of cause with effect. 'If we call gravity a power,' said Mayer, 'then we represent to ourselves a cause, which produces effects, without becoming itself diminished; we therefore conceive incorrect notions about the cohesion of the causes of things. Gravity is not a force,' he continued, 'but a property. Forces however are causes. Causes however are objects, which as regards quantity are indestructible, as regards quality are changeable. As soon as the idea of force is defined in this manner, it follows as a matter of course that no force can end in nothing; on the contrary the cause must be equal to the effect; there must be between the sum of the effects and the quantity of the cause a mathematical equation; and as every equation can be reversed, it follows that under suitable circumstances the cause can be reproduced from the effects.' Such were the principles upon which Mayer based the argument of his second publication. Besides this process of thinking, Mayer employed observation and experiment to support his hypothesis. He observed, thermometer in hand, the rise in temperature which takes place in the rotatory machines which tear the rags in paper-mills, and which in Germany, from their origin, are called 'Dutchmen.' In his consulting-room he shook a bottle containing a litre of water until its temperature had risen from  $12^{\circ}$  to  $13^{\circ}$ , excluding

the influence of the warmth of his hands by thick felt-gloves. This experiment made it to his mind a broad practical truth, that work could be transformed into heat. It has been said that the logical distinction between power and property, as regards the definition of gravity, was derived from Kant's critical inquiries. But the writer in the *Schwäbische Kronik* asserts that there was no indication in Mayer's writings of his ever having studied this author. He had no good opinion of what was then termed philosophy, particularly 'Naturphilosophie' in Germany, and avowed that the endeavour to penetrate into the mystery of the order of the world by means of hypotheses was a parallel to the endeavours of the adepts.

From 1842 to 1845 Mayer was incessantly engaged in the study of organic chemistry, particularly of the work of Liebig in which this science was for the first time systematically considered as to its applicability to physiology and pathology. The fruit of this study was the Essay 'On Organic Motion in its connection with the Chemical Change of Matter in the Body.' It is considered that this essay bears the stamp of the maturity of his genius; it is remarkable by the consciousness and simplicity of its enunciation. It contains such imperishable sayings as the following: 'There is but one force' 'In everlasting change it circulates in living and inanimate nature; in neither is there a process without a change of the form of force.' 'The science of physics has to investigate the metamorphoses of force as that of chemistry has to consider the changes of matter.' 'The creation or annihilation of a force can neither be effected nor conceived by man.' 'From nothing, nothing can come.' 'Nothing can come to nothing.' 'Chemistry teaches the fixity of the nature of the ultimate forms of matter; physics teach the fixity of the quantity of force, and the changeability of its forms.' 'Gravity, motion, heat, magnetism, electricity, chemical difference, are all only different forms in which appears one and the same force which governs in the universe; for every one of them can by proper arrangements be transformed into every other.' Elated by the view of nature which he has thus obtained he exclaims: 'We feel quite well that we are about to do battle with the most firmly rooted hypotheses which are canonised by the greatest authorities; that we desire to remove from natural



science in the shape of the so-called imponderables the last remnants of Greeian gods: but we know also that nature in its simple truth is greater and more glorious than any image made by man's hands, than any illusion of the created spirit.' This sentence is no doubt somewhat obscured by inappropriate adjectives, and therefore has been considered as tainted with mysticism. The writer in the *Kronik* supposes that under the category of the last remnants of the gods of Greece, Mayer had meant to include in the first place the so-called vital force. Against this no doubt he declared 'war' most directly. 'We must enter our protest,' said he, 'against the putting up of a particular vital force.' 'Bring into the juices of the body of the strongest and healthiest man a grain of putrid matter, and a decomposition will soon ensue to which neither nature nor art can put a limit, and which soon becomes fatal. Where in such a case is the vital force? where the power to make resistance to external causes of disturbances? *hic Rhodus, hic salta.*' Vital power with him resides in the sun. 'The sun is the constantly wound-up spring, which keeps the wheelwork of terrestrial action going. It is the light of the sun which, changed into heat, causes the motions in our atmosphere, lifts the waters up to the clouds, produces the streaming of rivers. Light, the most mobile of all forces, caught by the earth in the swiftness of its motion, is by the plants transformed into rigid shapes: for the plants on the earth produce constantly a quantity of chemical difference, they form a store in which the volatile rays of the sun are fixed and deposited so as to be fit for the subsequent use of man and animal. The plants take in a power, light, and produce a power, chemical difference. During the process of life there takes place only a transformation of matter as well as of force, but there is never any creation either of the one or of the other.' The truths contained in these sentences Mayer now proves more in detail. The sun-power stored in the plants is consumed by men and animals, passes into their blood, and is then changed by oxidation into heat; the heat thus produced in the body, serves in part to maintain the body at the temperature necessary for its existence, while another part is transformed into work. He then shows that the equivalence of heat to work obtains in the organic world as well as in the inorganic,



in which latter it has been first discerned. In the organic world also, the unit of heat is equal to the mechanical effect which a mass of one kilo produces when falling through a height of 425 metres. Starting from the results of Gay-Lussac concerning the expansion of a given quantity of air by heat, he arrives at certain conclusions concerning organic life. If air is warmed while its volume is constant, less heat is required than when it is heated to the same temperature under a constant pressure. In the first case the air is enclosed in a rigid sphere; in the second case it is enclosed in a vessel, in which on expansion it has to lift a weight; the excess of heat required in the second case as compared with the first (the degree of heat to which the air is raised being equal in both cases), is in the second case used to perform work, while in the first case no work is performed. These data he applied to the consideration of the results of the combustion of coal in a steam engine, and found that the work produced is only from 5 to 6 per cent. of the amount which the coal would be capable of producing if no effect was lost in the shape of heat: and further that the work produced in a cannon by the gunpowder averaged about 9 per cent. of the theoretical amount. The temperature (amount of heat units) which 95 parts of coal will produce when burned under an open boiler filled with a certain quantity of water, can be produced in a steam boiler doing work only by the burning of 100 parts of coal. The heat produced in a living organism is governed by the same law. A part of the heat, which is supplied by the combustion of the blood, is used up by the work which the organism does; from the amount of the work done by the organism, the amount of heat which is equivalent to it can be calculated according to the formula given. The working muscle requires more heat than the resting muscle; the parts of the body which do the least work in a mechanical operation secrete the most sweat; the forehead overflows with sweat, while the arms consume the heat produced by an excited circulation in the shape of work. For the production of a given mechanical effect (in or by the human body) a certain amount of heat has to be expended, which can be estimated theoretically beforehand, and this estimate is found correct by experience. Man and animals are subjected to the same laws as the steam-

engine, but man is the more perfect machine of the three, inasmuch as under the most favourable conditions, he is able to transform 20 per cent. of heat produced by the neutralisation of chemical difference into work. Mayer uses some illustrations which, if they are correct, are very interesting; such as that a smith, who could hammer the head of a nail until it became red-hot, effected this by transferring the heat of his arms to the head of the nail. Other illustrations given by Mayer we fear are difficult to prove as facts, though they probably assisted his argumentation in a measure.

The muscle is the tool by means of which the metamorphosis of heat into work is effected; but this metamorphosis is not effected at the expense of the muscle. This proposition Mayer illustrates by considering quantitatively the muscle of the heart and the work which it performs. And in the course of the considerations following upon this, he attempts an appreciation of the phenomenon, already mentioned as having been observed by him at Batavia, namely that the venous blood of the sailors whom he bled there, had such a remarkably light red colour. He says, from the established laws (of the metamorphosis of force) it follows with necessity, that the difference between the heat of the organism and the heat of the surrounding medium stands in a quantitative relation to the difference in the colours of the arterial and venous blood respectively. The greater the difference of temperature, or the production of force, the greater must be the difference in the colours; the smaller the difference in the temperature, the smaller must also be that in the colours. This difference in the colours is an expression for the amount of the consumption of oxygen, or for the strength of the process of combustion in the body.

In 1851 Mayer published a paper 'On the Power of the Heart' in *Vierordt's Archiv.*; and in 1862 a paper 'On Fever' in *Wunderlich's Archiv.*; the latter he termed an iatrophysical essay. It cannot be said that these papers have had any particular effect upon the consideration of the relative questions, but they aided in producing the knowledge, that organic life, as far as it is open to physical consideration, is amenable to the law of the mechanical equivalent of heat. He said that 'the

existence of an unchangeable quantitative relation between heat and work was a postulate of the physiological theory of combustion.' It cannot be doubted that he proved his proposition. The *Dynamics of Heaven* date from the year 1848. In this essay he applied his principles to the motions of celestial bodies, and came to results which constituted new prospects, new questions and answers, to astronomers; the beating of the pulse of the earth, ebb and flood-tide, shooting stars, the heat of the sun, were here subjected to investigation, and their explanation was more closely approached than had been done before. Many of Mayer's views have become obsolete by the rapid progress of science during the last twenty years, e.g. by spectrum analysis, of which he then knew nothing; other of his views, such as that the tides must delay the velocity of rotation of the earth and make the days longer has remained an admitted fact to this day. This result was not obtained by calculation but by deduction from his principle. Mayer opposed the hypothesis of Herschel concerning the existence of a photosphere of the sun, a hypothesis which at the time enjoyed almost universal acceptance. He contemplated the number of rays which the sun constantly sends out, and put the question by what means the sun could be able to replace this enormous loss of power. He answered it by his principle, namely that some kind of motion must be converted into heat. 'In the entire space of the solar system there rotate an incalculable number of smaller bodies; the comets, of which there are in space, according to Kepler's estimate, a greater number than of fishes in the sea; the shooting stars, fireballs or meteors, the sidereal dust in the zodiacal light; then there are all the planets; all these move in a resisting medium, the ether, and consequently their circuits become constantly more contracted, until they at last necessarily unite with the sun. Here their motion comes to an end, and, according to the law of the mechanical equivalent of heat, their motion must be changed into heat. The fall of a mass of asteroids into the sun produces an amount of heat which is from 4,000 to 8,000 times greater than the heat which an equal mass of coal would be able to produce by combustion. From this circumstance the heat of the sun is greater than any heat which can be imagined on earth, and the diathermic properties of the

sunbeam are greater than those of any heat beam produced on the earth. The objection which has been made to this theory, namely that if it were correct the volume of the sun must be constantly on the increase, is as to the fact correct; but the accession is so small that 28,500 years would have to pass, before the diameter of the sun increases by as much as a second of an arc. The heat of the sun is therefore maintained by the fall of inter-planetary matter upon the sun; all bodies which circulate round the sun have their cradle in the periphery, their grave in the centre. Even the solar spots, and the faculæ, or protuberances, receive an explanation by this hypothesis; the fiery ocean of the sun is excited down to low depths by the meteors which plunge into it, and its liquid and gases are projected upwards in the shape of glowing mountains.' This theory is nowadays not admitted as exclusively true, but the probability of the phenomenon cannot be denied; it must be admitted that heat of all degrees, to white heat, may be produced by the means referred to; Mayer's explanation is on the other hand strictly true of the fiery nature of meteors, shooting stars, and similar bodies. Their motion is in the resisting medium of the air transformed into heat.

The phenomena of the tides Mayer correctly estimated as regards one of their principal mechanical effects. He termed them an arrangement for breaking and retarding the velocity of the rotation of the earth round its axis. The earth rotates from west to east, the tides from east to west. Now if the proportions of land to water are estimated according to certain data, the tidal effect would be to prolong the day by one-sixteenth part of a second in 2,500 years. La Place has however proved that the length of the day has during the last 2,500 years not changed even to the extent of one five-hundredth part of a second. This does not prove, according to Mayer, that the retardation of the rotation of the earth does not take place, but only that there must be an action opposed to this retardation, which in its turn again accelerates the rotation, and this accelerating action he assumes, with other philosophers, to be caused by the slow constant contraction of the earth in consequence of its gradual cooling. These considerations led him to



the hypothesis that in the existence of the earth there must have been three distinct great periods; the first period characterised by a decrease of the length of the day in consequence of cooling and contraction of the earth; the second period in which this accelerating action of the contraction by cooling is counterbalanced by the retarding action of the tides; a period therefore in which the length of the day remains constant; and lastly the third period, in which the influence of the tides prevails over that of contraction and cooling, and in which therefore the length of the day increases. In 1870 Mayer gave a lecture on earthquakes, and in this he adverted to his theory of the effect of the tides, and stated, that with the aid of some observations of Adams of Cambridge he had come to believe, that the earth was already in the third period, in which after a time of equilibrium its age had begun to manifest itself.

The Essay which Mayer published in December, 1850, 'Remarks on the Mechanical Equivalent of Heat,' contains no new developments, but is a more complete and in some respects more correct account of his previous discoveries. He did not record any progress in this publication, but on the contrary began to become stationary while others progressed beyond him. For some had already then begun to look upon heat itself as a form or mode of motion. Against this idea he spoke decidedly, 'for,' said he, 'in order to become heat, motion, be it a simple one, or a vibrating one like light, or radiating heat, must cease to be motion.' His quasi fear to come to this result, is explained by some as arising from his want of acquaintance with the higher branches of mathematics. We may however be quite sure that the sense of solitude in which he found himself when apparently nobody took any heed of his work and his endeavours and ideas, and the depression which overpowered his intellect for long periods, had more to do with the arrest of his farther development than the want of higher mathematical knowledge.

It is perhaps good advice, that those who desire to study the science of physics as it is in our days, cannot do better than begin their studies with Mayer's works. They are written with a deep enthusiasm which engages one's sympathy, and with a poetical vigour which carries the heart as well as the intellect

with it. He carried his theory, says a writer, which he had formed from the heliocentric standpoint, to the widest spheres of application. He laid, through the secrets of the processes of the life of organised beings as well as through the wonders of the starry world, the even road which leads to the river of knowledge, and to its great fountain, thought.

## V.

ON THE COLOURING MATTERS IN THE SHELLS OF THE EGGS OF BIRDS; AN ILLUSTRATION OF CHEMICAL DIAGNOSIS AT A GLANCE, AND OF THE VALUE OF ABSTRACT CHEMICAL RESEARCHES. (*From the Pathological Institute.*)

IN 1858 W. Wicke, Professor of Chemistry to the Agricultural Institute at Göttingen, presented through Prof. Wöhler to the Academy of Sciences of that University a paper on the colouring matters of birds' eggs, which was probably the first chemical essay on that subject ever written, and was printed in the *Göttinger Nachrichten*, etc., 1858, p. 314. The author treated the shells with hydrochloric acid, water, and boiling alcohol in succession, and obtained a brown colouring matter, which he declared to be biliphæin, or brown colouring matter of bile, and a green pigment, which he declared to be identical with biliverdin, the green modification of the colouring principle of bile. He found also that the green colour of his extracts had a tinge of blue, or that some extracts might be more blue than green; and the brown extracts might appear yellow by dilution or other causes. The pigments were insoluble in water. They were deposited, according to him, on the outer layers of the shells, and he suggested that they were deposited in the cloacæ of the birds upon shells ready formed of white calcic carbonate in the oviducts. In support of that surmise he quoted a communication made to him by a sportsman, who had shot several birds, which ordinarily bring forth coloured eggs, in the oviducts or cloacæ of which there were contained eggs, either quite colourless or only faintly spotted, as if they were in process of being spotted with the pigment or pigments.

The subject was next treated by Sorby, in a paper which was

printed in the *Proceed. Zool. Soc. Lond.* for 1875, p. 351. In this research the spectroscope was applied to the matters in question, and peculiar spectra were discovered, of which the author soon found the meaning. But this he explained away by a laborious argument. He distinguished a red, a blue, and a green pigment, and other little defined products, and added little to their chemical description or diagnosis.

Three years later C. Liebermann (*Ber. Deutsch. Chem. Ges.* 11, 1878, 606) again studied the subject, being acquainted with the essay of Wicke, but evidently not with that of Sorby. He found the colours of birds' eggs referable to two matters, one blue or green, which he defined with confidence as a biliary pigment, and another not recognised as to its origin, but distinguished by the characteristic spectrum described by Sorby. He treated the eggs with acid, and separated flakes which were mostly coloured green. Alcohol produced coloured solutions, which were rarely sky-blue (*Turdus musicus*, *Sturnus vulgaris*, *Sylvia phœnicurus*, *Ardea argentea*), more frequently green (*Corvus corone*), without fluorescence; very frequently bluish-green, with strong blood-red fluorescence (*Larus canus*, and *ridibundus*, *Sterna hirundo*, *Scolopax*, *Hematopus*, *Stringa*); in the smallest number of cases faintly reddish, fluorescent, with a feeble touch of green (*Falco tinnunculus*, *Sylvia hypolaris*, *Tetrao islandicus* and *coturnix*, *Fulica atra*). He used the shells of peewit's and gull's eggs to produce pure pigments, but obtained only dark green smeary masses. He then described the peculiar absorption spectra of the colouring matters, as observed by Sorby, and found that they differed according to whether they were contained in a strongly acid, or in a feebly acid, or in an alkaline solution. He gave long details concerning these transformations. He found some of the pigments soluble in chloroform. As he obtained Gmelin's reaction (the changes of colours with nitroso-nitric acid) with the green (not with the red) pigment, he believed the green to be biliary, but he was not certain that it was biliverdin. He doubted Wicke's opinion of the brown pigment being biliphæin or bilirubin, but got entangled in a net of errors when he endeavoured to attack the problem by means of the supposed oxidising action upon bilirubin of



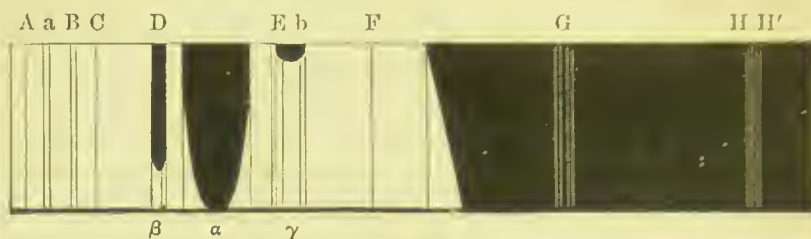
bromine. He adopted the opinion of Wicke, to the effect that the colours were imparted to the eggs in the cloacæ, and not in the oviducts.

I recognised at a glance the identity of the spectra described and figured by Sorby and by Liebermann with those characterising *cruentin*, a derivate of the colouring matter of blood (hemochrome), discovered by me before 1866, and first described and figured in the *Tenth Report of the Medical Officer of the Privy Council*, Appendix 7, pp. 227–232. From this research I give the following extract, with the diagrams of the spectra belonging thereto, as the data apply, *mutatis mutandis*, exactly to the phenomena exhibited by solutions of the red colouring matter of birds' eggs, *ovocruentin*, as I propose hereafter to name it.

*Cruentin, a new derivate of hemato-crystallin, and of hematin.*—When human or animal hemato-crystallin (hemochrome) is boiled with sulphuric acid, it becomes chemolysed, the albumen dissolves and yields its particular products, a portion of the hematin formed also dissolves and colours the fluid ruby red, while a brownish red grumous matter remains suspended in the fluid in an insoluble state. This is a mixture of neutral cruentin with its sulphate. By washing with water this matter loses sulphuric acid and becomes ultimately free from it. Treated with sulphuric acid it dissolves completely, and is now sulphate of cruentin. Hematin treated with sulphuric acid also yields cruentin.

*Cruentín sulphate.*—When a concentrated solution of this body is examined with the spectroscope, it shows one broad black band in red to orange. The visible blue is about as extensive as the visible green. On dilution this band splits up into two, and a third very feeble band in green becomes visible just to disappear on the slightest dilution. We have thus a spectrum of three bands represented by the following diagram, in which the absorptions, carefully measured by the spectrometer, are shown in their positions relatively to the sun-lines, and the intensities of absorption are expressed by curves enclosing the black spaces indicating the absorptions. The order of intensity of these bands is not the same in all dilutions. There is a point, when  $\gamma$  is just visible, intensity 1

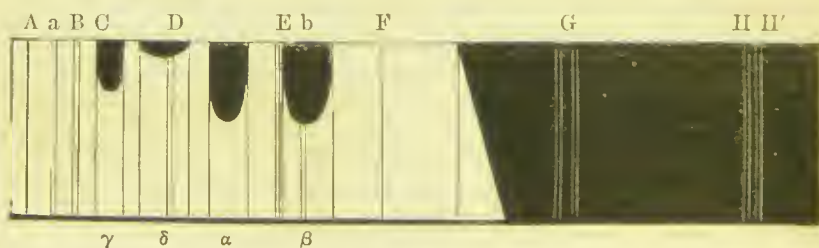
(I distinguish ten different degrees of intensity, expressed in diagrams by ten different lengths of the band shadows beginning



*Diagram of Spectrum of Sulphate of Cruentín.*

always at the upper margin of the diagram and reaching perpendicularly downwards), at which  $\alpha$  and  $\beta$  are of equal intensity, while just before the dilution at which  $\gamma$  appears,  $\beta$  is decidedly most intense. On continued dilution, however,  $\beta$  disappears before  $\alpha$ . The absorption is at all times equal to that of a solution of blood in water of equal intensity of colour as seen by the eye, so that when the fluid shows the merest tinge of pink, the bands are yet clearly visible.

*Neutral fluorescent cruentín.*—When the insoluble residue from the sulphuric acid treatment is washed to neutrality and dried, a portion of it is soluble in ether and chloroform. The ether solution has four bands which are a little paler than, but in situation as nearly as can be identical with the bands of the chloroform solution. This latter, prepared by boiling, is dark red, and shows the following spectrum.



*Diagram of Spectrum of Neutral Fluorescent Cruentín.*

The saturated chloroform solution was one ctr. thick. The spectrum was measured with the aid of gaslight only. It fluoresced with a splendid blood-red colour in the cone of sun-rays produced by a lens. This is the first body which is known

to fluoresce with homogeneous light, that is to say, the same kind of light or colours which it transmits. The fluorescent cone has an intensity of colour equal to that of a solution about ten times more concentrated. Considering its beauty alone the phenomenon may be placed by the side of those exhibited by quinine, chlorophyll, or eudbear. But by its peculiarity it is perfectly unique.

We shall see below how this description applies word for word to oocruent in.

*Alkaline four-banded cruent in.*—The spectrum of this form is represented by the following diagram :

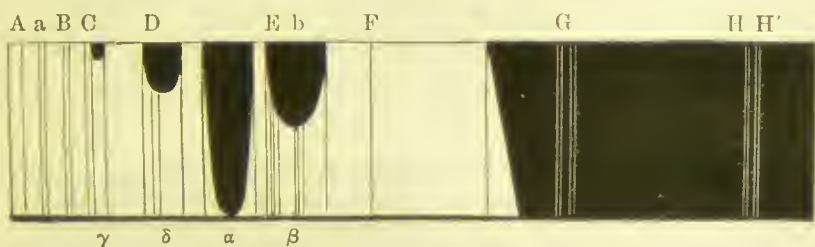


Diagram of Spectrum of Alkaline Cruent in.

*Neutral five-banded cruent in in alcohol.*—When the preparation from which chloroform extracts the neutral fluorescent cruent in is treated with alcohol, it dissolves easily and almost entirely: The concentrated solution allows a little red to pass ; on further dilution three bands appear, ultimately five, one in red feeble, one in yellow also feeble, both narrow, and three dense and dark bands in green, the last one situated at the point of transition into blue.

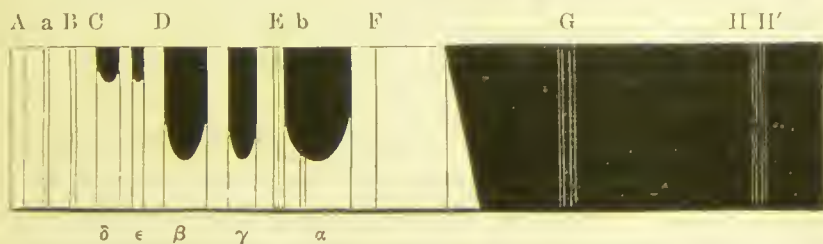


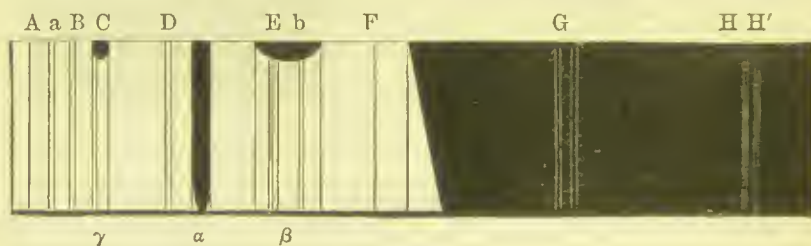
Diagram of Spectrum of five-banded Cruent in.

When this solution is made alkaline with ammonia, the band  $\epsilon$  disappears, and the others arrange themselves as stated above under alkaline cruent in.

The five and four-banded cruentin are both transformed into the three-banded acid cruentin by solution in sulphuric acid. On addition of water the five-banded cruentin is again obtained, which by ammonia becomes four-banded.

The five-banded cruentin was obtained by extracting with ether and much glacial acetic acid, cruentin many times dissolved in sulphuric acid and reprecipitated by water. Immediately on the acid being washed out by water one band in orange disappeared, the red was more intense, and the ether solution showed the four-banded cruentin spectrum.

*Reduced cruentin.*—Cruent in dissolved in ammonia shows the four bands. When an ammoniacal tartrate solution of iron sub-oxide is added to it, it changes its spectrum in the following manner. There is a pale band  $\gamma$  in red, there is a prominently



*Diagram of Spectrum of Reduced Cruent in.*

dark  $\alpha$  in green, and a broader but paler one  $\beta$  in green towards blue. On being shaken with air the solution becomes much lighter, and presents again the spectrum of four-banded cruentin.

When the solution of reduced cruentin is acidified with sulphuric acid, it yields a precipitate; after filtration it is much lighter and shows the spectrum of cruentin in the dilution in which  $\gamma$  has completely disappeared. Cruent in therefore exhibits the peculiar property shown by hemochrome and hematin, that it can be oxydised (in alkaline solution) and reoxydised (in alkaline or acid solution). During this process however much colouring matter is lost by changes not yet scrutinised. In its alkaline solution it is deoxydised and reoxydised as easily as hemochrome (hemato-crystallin). It is most remarkable, physiologically, and particularly with regard to the occurrence of this matter in eggshells, that a decomposition product of



hemochrome of the second order retains what I will term the breathing power of the blood-corpuscle.

By treatment with aqueous hydrochloric acid and warming, cruentin undergoes a permanent change, which does not permit the four bands of the alkaline solution to be reproduced by alkali. This change is described on pp. 231 and 232 of the essay quoted, and further followed out in a subsequent essay.

For our present purpose the data given above will suffice, for they will enable us fully to diagnose and identify the red or brown colouring matter of the shells of birds' eggs.

*Experiments and observations on the shells of birds' eggs.*—Some, commonly termed plover's eggs, were freed from their contents, washed with water and treated with dilute hydrochloric acid. When the effervescence had subsided and after the mixture had stood many hours, the shells were transformed into brownish green membranes, and the solution was light violet red, indicating that an excess of hydrochloric acid had been used. Placed in the spectroscope it showed the spectrum of *acid cruentin* in a beautifully clear manner, blue and violet being very transparent. (With the sulphate blue and violet are much more absorbed.) The addition of ammonia to this solution caused a rust-coloured precipitate of five-banded cruentin. The precipitate could be passed through all the phases of the reactions mentioned above. It was soluble in ether and chloroform; and the latter agent even extracted the acid cruentin hydrochlorate from the acid watery solution.

The coloured membranes of the eggs, after complete washing out of all hydrochloric acid by means of water, were treated wet with alcohol (drying was found to be destructive of a great part of the pigments) and gave a deep green solution. This before the spectroscope showed a considerable continuous absorption of the red, effected no doubt mainly by the green ingredients of the solution, but in part also by the absorption in red of five-banded cruentin, of which the three principal bands,  $\beta$ ,  $\gamma$ ,  $\alpha$ , occupied the same positions as the three bands produced by the suspended precipitate of five-banded cruentin from the acid watery solution just described. The alcoholic solution was therefore a mixture of the solutions of at least two bodies, the green pigment and neutral five-banded cruentin. The process

by which this result had been obtained indicated a way in which the green and red colours could be separated, namely by exhaustion of the shells with very dilute hydrochloric acid, in which only the calcic carbonate dissolved, while the green and the red pigment remained insoluble. After removal of all acid by water, the green pigment, ovoviridin, is soluble in alcohol; the red pigment still remains insoluble in neutral alcohol, and dissolves only in the presence of an excess of hydrochloric acid. When the two pigments are once together in alcoholic solution, a separation of one from the other becomes extremely difficult.

I now treated a large number of birds' eggs (peewit, moor-hen, thrush, blackbird, starling, fieldfare, hedge-sparrow, and others) in the same manner as those of the peewit above described. The acid watery filtrates from the shells of eggs of thrushes and blackbirds were slightly green. The alcoholic extracts of most membranes were deep green, those from the shells of the moor-hen alone were greenish yellow. The alcoholic solution on addition of ammonia gave a precipitate. Four eggs of fieldfares (which are buff coloured with reddish spots) gave as much ovocruent in solution as sixty thrushes' eggs (which are blue with dark spots). Blackbirds' eggs also gave a deeper coloured hydrochloric acid solution than thrushes' eggs.

An experiment was made in which it was attempted to extract the lime from the eggshells by acetic acid. But this miscarried entirely, all the colouring matters being altered or transformed into widely different products.

The hydrochloric acid solutions from many eggshells were evaporated, and formed a precipitate during concentration of neutral five-banded cruentin. The solution or filtrate was dark brown, and exhibited the spectrum of acid two-banded cruentin. It was precipitated with ammonia and the precipitate was filtered off; this precipitate gave a portion to concentrated hydrochloric acid in the cold, which was isolated; the residue insoluble in hydrochloric acid was treated with slightly acidified boiling alcohol and dissolved, forming a splendid red coloured solution of fluorescent cruentin, with all features of spectrum and reaction described. A small residue insoluble in alcohol dissolved in concentrated hydrochloric acid, and was cruentin hydrochlorate.

There cannot therefore be the slightest doubt of the red colouring matter being identical with cruentin as described by me. The spectra of analogous solutions were compared by projection through the same slit and the same prisms, and found to coincide completely with each other.

*The green pigment.*—Its alcoholic solutions are at first difficult to filter. They can be evaporated and leave the pigment as a green mass, which is however now no longer entirely soluble in alcohol. The residue is insoluble in hydrochloric acid; it is partly soluble in sodic carbonate, partly insoluble. When the sodic carbonate solution is evaporated to dryness, and the pigment set free by acid, it is now insoluble in alcohol. In short the green pigment undergoes a continuous change by almost every kind of treatment.

The green pigment is therefore much less soluble than biliverdin; it resembles however biliverdin, not only by its solubility in alcohol, and green colour, but also by giving Gmelin's reaction, the transformation colours, blue, green, red, orange, yellow with nitroso-nitric acid, whether in alcoholic solution or in the dry state.

When the green pigment in alcohol exhibits a blood-red fluorescence (in which state it resembles closely a solution of chlorophyll so called), it is mixed with fluorescent cruentin, to which latter the fluorescence is due. It then gives the cruentin spectrum, while when pure it gives no detached bands, but only a continuous absorption on the side of the red.

I have sometimes obtained a sky-blue or blue solution from eggshells directly, by the simultaneous action of hydrochloric acid and alcohol combined; but I cannot assert the presence in many eggs of a blue colouring matter. From peewit's eggs I once obtained a bluish-green extract by ether and acetic acid; the ether solution lost all green or blue colour on standing, and retained only a slight pink colour; it had however a great power of rose-red fluorescence.

The green pigment is not soluble in chloroform, or in ether. Some eggshells after extraction with hydrochloric acid, were extracted with alcohol, and the solution was evaporated. During condensation the solution, as is always the case, lost its primitive brilliant colour and became cloudy and formed a deposit. The

latter was removed by filtration, and the filtrate evaporated to dryness. The residue was again extracted with absolute alcohol, and the filtrate again evaporated to dryness. (What the alcohol left undissolved was partly primary impurity from the membranes, partly changed pigment.) The residue was now exhausted with ether, which dissolved a brownish red matter, mainly cruentin, and left a dark green matter undissolved, which was now entirely soluble in alcohol.

The green pigment is therefore similar to, but not identical with biliverdin. It is, like the ovocruent in, a product of the villous appendages of the inner surface of the oviducts of the birds which produce coloured eggs. The idea that it might be deposited on the eggs in the cloaca, from the intestinal contents carried that way, that it might in fact be the result of a mere soiling with faecal biliary matter, is too improbable in itself, and is contradicted by too many data of a very certain nature.

In the first instance the cloaca never contains faeces when an egg passes through it; white eggs are never soiled with faeces in the cloaca even partially. Secondly, the pigments are imbedded in the layers of chalk which form the shell, and not only so to say painted on the outside. Further, the pigments are several, and of these ovocruent in does not occur in the faeces; further, some eggshells are uniformly impregnated with a red or brown, while others are uniformly impregnated with a green or blue pigment; others again are uniformly blue, but spotted with red or brown patches, and so on. These varieties show that the pigmentation of eggshells is the work of specific organs, and these organs must needs be glands distributed in various orders in the oviduct; the oviduct is the organ in which the shell is made from bicarbonate of lime secreted by the glands in its limiting membrane; in this chemical laboratory is the natural place for glands, which have the power to split up hemochrome, and deposit the pigment products on the structure which is so rapidly built up within its cavity.

Although more than a thousand eggshells were used to extract the pigments which I have described in the foregoing, the material obtained was insufficient to enable me to ascertain the chemical composition of the products by elementary analysis. It is therefore evident, that the question of the composition of



these pigments could not easily be decided with the aid of materials derived from them directly. Under these circumstances we may therefore, at least as regards ovocruent in, fall back upon cruent in made from blood by chemolysis, and use it for the purpose indicated. It is at all events clear, that an abstract research, like that on cruent in, has produced results, which have enabled us at a glance to determine the nature and physiological origin of an interesting phenomenon of the animal world, and to eliminate several serious errors with which the physiological question had been surrounded. The pigmentation of eggshells is perhaps not to be considered as an important biological problem, but, as has been shown by an author in the German periodical devoted exclusively to the promotion of the philosophy of evolution, it is one of the conditions of existence and continuance of living beings, and as such is deserving of consideration.

In the placenta of several kinds of mammals colouring matters are deposited, e.g. in that of the dog; these too are of two kinds—green and red, and the green has ere this been held to be identical with biliverdin. In any case the analogy of this fact to that observed upon eggshells cannot escape attention. It involves no great stretch of the imagination to suppose that both occurrences have the same evolutionary origin. In other words, the pigment glands of the oviducts are remnants (or beginnings) of the villous coat of the uterus, the pigments are direct decomposition products of blood; the blood does not serve for the nutrition of a foetus, but only furnishes a tribute of respect, an ornamental or useful covering to the outside of the kind of sequestered being, with which it had in anterior stages of development more intimate relations, or with which it may have them in the future if the bird is in process of evolution towards a mammal.

## VI.

ON THE ORGANIC ACIDS OF THE BRAIN, WITH SPECIAL  
REFERENCE TO THE NATURE OF THE LACTIC ACID  
CONTAINED IN IT. (*From the Pathological Institute.*)

*Introduction.*—The organic acids of the brain are obtained as ingredients of the so-called water-extract, that is to say, the watery solution of alkaloids, carbohydrates, acids, organic and inorganic salts, which is obtained when the spirit-extract of brain is gradually freed from spirit by distillation, and from the phosphorised, nitrogenised, and cholesterous principles by deposition and filtration. When a general analysis of these extracts is intended, it is perhaps most convenient to remove the alkaloids in the first instance by special precipitants such as phosphomolybdic acid, phosphotungstic acid, or mercuric acetate. After removal of the excess of the reagent employed, inosite may be precipitated in the usual manner, and after the removal of the excess of lead, the fixed acids may be extracted by ether. When it is however intended to extract the fixed organic acids only, they may be extracted from the original water-extract by ether after addition of an excess of sulphuric acid. In the following processes alcohol was employed at a certain stage to isolate amido-acids and nitriles (leucin and tyrosin, etc.). Anyone who will examine the water-extract of brain according to this method will not leave much in the mother liquor to be accounted for. When pure phosphomolybdic acid is employed, the ultimate mother liquor may be incinerated and used for the analysis of the fixed alkalies and earths.

*Treatment of the water extracts of the Human Brain after removal of Alkaloids.* 1. *Extraction of Formic, Lactic, and Succinic Acid.*—After the bulk of the alkaloids had been removed from the extracts by mercuric acetate the filtrates

from the mercuric precipitates were freed from mercury by hydrothion, and evaporated to a syrupy consistence; the syrup was treated with absolute alcohol, and thereby separated into a portion soluble, and another insoluble in alcohol.

*Portion soluble in alcohol.*—The alcohol was evaporated, the residue treated with neutral plumbic acetate. The precipitate consisted mainly of phosphate and chloride, and was removed. After this, basic plumbic acetate was added to the filtrate, and the precipitate collected and decomposed with hydrothion: the concentrated filtrate from the plumbic sulphide on treatment with absolute alcohol gave a crystallisation of *inosite*.

The mother liquor of this inosite, freed from alcohol by evaporation and acidified, gave a small precipitate with phosphomolybdic acid, thus proving that the mercuric acetate had not removed all the alkaloid, but left some, which was precipitated by basic lead acetate (*third alkaloid*).

The lead was now removed from the mother liquor by an excess of sulphuric acid, and the acid liquid extracted with large quantities of ether at intervals. The ethereal extracts, after removal of the ether by distillation, left a mixture of acids (see below, § 4); of these, acetic acid, introduced with the mercury and lead salts, was evaporated on the water-bath; the remaining syrup consisted mainly of *lactic acid*, but also contained some *succinic acid*, as will be described more fully lower down.

2. *Leucin and Allied Bodies; Tyrosin.* *Portion insoluble in alcohol.*—This on solution in a minimum of water retained a quantity of *solid matter* in suspension which was separated by filtration; the residue on the filter was pressed, and on treatment with cold water gave to this solvent a matter recognised as *leucin and allied bodies*; the matter insoluble in cold but easily soluble in hot water, was *tyrosin*.

The leucin was purified by precipitating the coloured impurity out of its watery solution by means of mercuric nitrate, removing the excess of mercury by hydrothion, evaporating the solution, precipitating the acid liquid by ammonia, collecting and pressing the precipitate in bibulous paper, and recrystallising it from spirit. The first crystals were pure leucin;

the second crystals were however a different body, more soluble than leucin, and not easily separated from leucin on account of the similarity of properties; the last mother liquor dried up leaving a trace of matter.

The tyrosin was purified by hydrochloric acid and charcoal, and precipitation with ammonia.

The mercuric nitrate precipitate from leucin was also decomposed, and seemed to contain only a small amount of alkaloid No. 2.

3. *Peculiar Potassium Salt*.—The liquid part of the portion insoluble in alcohol was diluted with water, and treated with neutral lead acetate; the precipitate, decomposed by hydrothion, gave a syrup, which, treated with absolute alcohol, deposited a peculiar *viscous colourless potassium salt*; this was isolated, and on account of its peculiar nature the presence of the potassium was specially proved by combustion and platonic chloride. The part of the syrup soluble in alcohol was not further examined. The filtrate from the neutral lead acetate precipitate was treated with basic lead acetate, and the new precipitate decomposed with hydrothion; the concentrated product was treated with absolute alcohol, and gave white inosite, partly anhydrous, partly hydrated. In the mother liquor of this inosite nothing apparently but some alkaloid No. 2 remained. The mother liquors from which the foregoing bodies had been extracted and precipitated were freed from the impurities introduced as reagents as far as possible and evaporated, and then formed a nearly colourless, viscid, uncrystallisable mass; this was distilled in superheated steam, but yielded no glycerine. It gave a precipitate with phosphomolybdic acid, from which a syrupy alkaloid, smelling like sperma, was obtained (*see* alkaloid No. 3).

4. *Proceedings for the separation of the acids isolated by means of Ether*. *Lactic Acid*.—The mixed acids were heated on the water-bath until acetic acid was expelled, redissolved in water, and neutralised while hot with freshly-prepared zinc carbonate. The zinc salt was crystallised and recrystallised an indefinite number of times until perfectly white, crystallised throughout, and homogeneous. During these operations a coloured matter became insoluble, and had to be removed by



repeated filtration. The crystallised salt was found to be pure zinc lactate, containing the variety of lactic acid known as lactic acid from flesh, or sarkolactic acid.<sup>1</sup> Not only did the salts yield the particular amount of water of crystallisation which distinguishes them from the zinc salts of the fermentation lactic acid, but the free acid itself showed the power of polarising light, which is not possessed by the product of the fermentation of milk.

5. *Summary of analyses of Zinc Lactate from Human Brain.* Water of crystallisation.—Preparation I. (1) 0.6002 grm. dried *in vacuo*, on drying at 103° to 110° lost 0.0730 grm. water = 12.82 per cent.

Preparation II. (2) 1.6540 grm. which lost nothing *in vacuo* over  $H_2SO_4$ , dried at 105° to 108° (9 hrs.) lost 0.2135 = 12.90 per cent.  $H_2O$ . (Theory 12.82 per cent.  $H_2O$ .)

*ZnO in anhydrous salt estimated by ignition.*—Preparation I. (1) 0.2378 grm. left 0.0785 grm. ZnO, equal to 33.01 per cent. ZnO. (33.38 per cent ZnO required.) [Zinc-stains in crucible explain loss. The mode adopted by Wislicenus of determining Zn in lactate by combustion in platinum crucible, as he expressly says, is thus shown not to be very good.]

*Zn in hydrated salt, dried over  $H_2SO_4$  in vacuo, by precipitation with carbonate, &c.*—Preparation II. (1) 0.4897 grm. gave 0.1418 ZnO, equal to 28.9 per cent. ZnO, or 23.24 per cent. Zn metallic.

<sup>1</sup> Professor Gscheidlen of Breslau has published a paper in *Archiv Physiol.* 8 (1873) 171, entitled, 'On the Chemical Reaction of the Nervous Central Organs'; p. 178 he examines the acid which causes the acid reaction of the grey matter, and finds it to be lactic acid. Müller had already stated that the lactic acid in the brain was zymo- and not sarko-lactic acid. Gscheidlen supports this view on the basis of a single quantation of water of crystallisation on one decigramme of calcic lactate. He operated on the brain of dogs, and of a horse. He might therefore maintain that dog and horse had differently from man and ox ordinary lactic acid, and not flesh-lactic acid in their brain, if he had really proved that his one decigramme of salt was ordinary lactate. But one quantation of water of crystallisation on a decigramme of salt can prove nothing, particularly in so delicate a question. And further, the calcium salt of lactic acid is the least suited to decide this matter, as its hydration (in different preparations of sarkolactic acid) changes apparently capriciously, as will be shown below. In fact the statements of Müller, Gorup-Besanez, and others, and lastly of Gscheidlen, concerning the nature of the lactic acid from brain are proved to be erroneous.

(2) 0.5415 grm. gave 0.1571 ZnO, equal to 23.28 per cent. Zn.

Theory requires 23.35 per cent. Zn.

6. *Summary of analyses of Zinc Lactate from Ox Brain.*  
*Water of crystallisation.*—Preparation I. (1) 0.3546 lost 0.0454, equal to 12.80 per cent. H<sub>2</sub>O.

*ZnO in anhydrous salt estimated by ignition.*—Preparation I. (1) 0.1591 gave 0.0524 ZnO, equal to 32.93 per cent. Theory required 33.38 per cent. The loss explained by method as in case of human lactate above.

*Zn in anhydrous salt estimated by precipitation and ignition.*—Preparation II. (1) Dried at 105°, 0.2174 gave 0.0723 ZnO, equal to 26.69 per cent. Zn.

(2) 0.1440 gave 0.0479 ZnO = 26.69 per cent. Zn.

Theory requires 26.79 per cent. Zn, in the anhydrous salt C<sub>6</sub>H<sub>10</sub>O<sub>6</sub>Zn; the hydrated salt Zn (C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>)<sub>2</sub> (H<sub>2</sub>O)<sub>2</sub> contains 23.35 per cent. Zn and 12.82 per cent. H<sub>2</sub>O.

7. *Physical peculiarities of the Lactic Acid from Brain and its Zinc-salt.*—When the lactic acid as obtained from the ether extract, a state in which it was yet yellowish and gave out an odour, was decolourised by animal charcoal, and a somewhat concentrated solution of it was placed in a tube of 220 m.m. in length, and containing about 26 c.c. of fluid, and subjected to the influence of the polarised ray of yellow light in a Wild's polaristrobometer, it was found to turn the plane of polarisation to *the left* (to the measured extent, in the particular instance of an acid of uncertain strength, of 1° 20').

The acid was next transformed into zinc salt by boiling with zinc oxide, and the solution of salt was evaporated to the same volume as that occupied by the free acid. It now turned the plane of polarisation still to *the left*, but to the extent of 3° 15'. Thus the rotation from 0 to the left had been much increased, more than doubled, by the introduction of the zinc and the attendant thermal and hydric operations.

The zinc salts, which had been obtained in a state of purity, as proved in the previous paragraph, were dissolved in water and decomposed with hydrothion; the free acid was concentrated and became a colourless syrupy liquid; in this state it was not perfectly brilliant, but had a slight haze, probably from a trace

of finely divided sulphur. It was therefore allowed to stand for two months in a quiet place, and when the trace of particles had completely deposited, the clear part was isolated by decantation.

This acid, on being placed in a tube of 100 m.m. in length into the polaristrobometer, now turned the plane of polarisation *to the right*, in the particular instance of an acid of uncertain concentration to the extent of  $2^{\circ} 17'$  (average of seven observations).

It will be observed that I have made no attempt to determine the specific rotating power of any of the products obtained in these researches on lactic acid from the brain. The reason for this is the circumstance first observed by Wislicenus, that the rotating power of sarkolactic acid changes under a great number of influences, such as heat, water, and time. Thus free sarkolactic acid when dried over sulphuric acid *in vacuo* during twenty-one months is transformed into a mixture of anhydride,  $C_6H_{10}O_5$  (84 per cent.) first with lactic acid,  $C_3H_6O_3$  (16 per cent.), afterwards with lactide,  $C_3H_4O_2$  (16 per cent.). The solution of this mixture turns the plane of polarised light to the left,  $(\alpha) = -85.93^{\circ}$ . It is probable that all three products on treatment with water are transformed back into sarkolactic acid.

The watery solution of sarkolactic acid as ordinarily obtained, directly after extraction and concentration, shows a considerable polarisation to the left. This power is suddenly and greatly diminished after every addition of water or of spirit, but on standing it rises again, without however reaching its former value. The diminution of specific rotatory power by dilution is the greater, the more concentrated was the solution used for dilution, that is to say, the greater was the dilution in proportion to the strength of the original solution. These changes are due to the presence of anhydrides and lactide. And as every preparation of sarkolactic acid contains these anhydrides, according to Wislicenus, pure sarkolactic acid, as a preparation, does not exist, and therefore its specific rotatory power, which this author surmised to be to the right, cannot be accurately determined.

I have shown above that pure sarkolactic acid prepared from the zinc salt turns the plane of polarised light freely *to the right*. But the solution of the zinc salt from which this acid was pro-

duced, turned energetically *to the left*. The saturated normal solution of zinc salt turns the plane of polarised light steadily  $7^{\circ} 7'$  to the left. In oversaturated solutions the turning faculty is not increased, as might be supposed, but on the contrary is diminished.

The polarising faculties of sarkolactic acid, its hydrate, zinc salt, and anhydride may be described as follows:

Turning farthest to the <i>right</i>	. $C_3H_5O_3$ .
Turning less far to the <i>right</i>	. $C_3H_5O_3 + H_2O$ .
Turning least to the <i>left</i>	. $2(C_3H_5O_3)Zn$ .
Turning more to the <i>left</i>	. $2(C_3H_5O_3)Zn + 2H_2O$ .
Turning farthest to the <i>left</i>	. $C_6H_{10}O_5$ .

8. *Peculiarities of the Calcium Salt of Lactic Acid from Human Brain*.—This salt was made from pure lactic acid obtained as above described. Its solubility in water was so great that attempts at its crystallisation from this solvent were foiled by the solution setting to a solid mass. It was consequently recrystallised from strong spirit. When dried in air it was a light voluminous spongy mass of crystals.

*Analyses:*

(a) 0.7210, on drying at  $105^{\circ}$ , lost 0.1531  $H_2O$ , leaving 0.5679 of anhydrous salt; consequently water of crystallisation = 21.2 per cent.

(b) 0.7010 gave 0.3385  $CaSO_4 = 0.0995$  Ca, or 14.20 per cent. Ca.

(c) 0.4678 gave 0.2240  $CaSO_4 = 0.0658$  Ca, or 14.08 per cent. Ca.

These data do not correspond to any of the recorded data concerning this salt, which recorded data themselves differ from each other, or on the assumption that there was only one hydrate, contradict each other. It is now assumed by some that the sarkolactate of calcium as commonly obtained has the formula:

2 Ca, $4(C_3H_5O_3) + 9 H_2O$ , water	. . . = 27.09 per cent.
Formerly however a hydrate was mostly described containing water	. . . = 24.83 per cent.
The hydrate just described contains	. . . = 21.2 per cent.



Now the second salt corresponds to one with four molecules of water of crystallisation, while the last leads to no even proportion between salt and water of crystallisation, but is intermediate between the salt containing four molecules and a hypothetical salt containing three molecules of water, which requires =19.89 per cent.

The new salt must therefore be considered either as a compound or mixture in nearly molecular proportion of the salt, containing 24.83 per cent., with the hypothetical salt containing 19.89 per cent. of water, or in more simple terms as a salt consisting of two molecules of anhydrous lactate and seven molecules of water of crystallisation, requiring 22.35 per cent.  $H_2O$ ; possibly a lower homologue of the salt with nine molecules of water. The depression of the water by mere admixture of anhydride was improbable owing to the uniform character of the crystallisation.

I am therefore of opinion that there are at least three, if not four, different crystallised hydrates of calcic lactate, and that the amount of the hydration is probably dependent upon the concentration of the solution, if it be a watery one, or upon the aqueosity of the solvent, if it be spirit.

9. *Note on the Theories concerning the Chemical Constitution of the Isomeric Lactic Acids.*—The lactic acid specimens obtained from different sources behave as if they were one and the same body. But on further inquiry, particularly by means of decomposition and the analysis of calcium and zinc salts in their hydrated state, it is found that the lactic acid obtained by the fermentation of different sugars differs from the lactic acid contained in the flesh and brain; it is further maintained that the acid obtained from flesh consists actually of at least two lactic acids; and that in addition there is a fourth lactic acid obtained by chemical synthesis. The lactic acid of fermentation can be produced from the lactic acid from flesh. We have therefore here to deal with an important case of isomerism, of which at least two terms were believed to be well understood. The fermentation lactic acid was said to contain the radical ethylenc, and was therefore also termed ethylene-lactic acid; whereas the flesh or sarkolactic acid was said to contain the radical ethylidene, and was termed ethylidene-lactic acid. The

acid  $C_3H_6O_3$ , obtained from glycerin-iodopropionic acid, termed by Heintz ethylene lactic acid, although isomeric with lactic, had better be termed hydracrylic acid because on thermolysis it decomposes, yielding water and acrylic acid. (Wislicenus, *Ann. Chem.* 166, 1873, 3.) By oxydation with silver oxyde it yields carbonic acid, probably carbacetoxylic, glycolic, and oxalic acid, but no glyceric or acetic; by fusion with caustic alkali, formic and acetic acid. According to Wislicenus, the synthetical ethylene lactic acid, produced from ethylene cyanhydrine, can be obtained pure with very great difficulty only, as its salts scarcely crystallise; and according to him also, this acid is identical with the second lactic acid, occurring in smaller quantity in the mixture of two lactic acids hitherto termed sarkolactic acid.

The lactic acid which occurs in flesh in the largest quantity, and yields a zinc salt with two molecules and a calcium salt with  $4\frac{1}{2}$  molecules of water of crystallisation, is, according to Wislicenus, ethylidene lactic acid. Heintz agrees in this conception in so far that he admits a part of the sarkolactic to be ethylidene lactic acid; but another part he assumes to be (what he terms ethylene lactic acid, namely) the above-described hydracrylic acid. (*Ann. Chem.* 157, 1871, 314.)

The four assumed isomers of the formula  $C_3H_6O_3$  are therefore (1) fermentation lactic acid, also termed ethylene lactic acid; (2) principal flesh lactic acid, also termed ethylidene lactic acid; (3) second flesh lactic acid, or synthetical ethylene lactic acid; (4) hydracrylic acid, termed by Heintz ethylene lactic acid.

Erlenmeyer (*Ann. Chem.* 158, 262) could not confirm Wislicenus and Heintz as to the invariable existence of two lactic acids in the sarkolactic acid, and explained the results of these authors as possibly caused by an oversaturation of the solution of the zinc salt, to which he shows the compound to be prone. He never obtained malonic acid by oxydation of sarkolactic acid (which had been so obtained by Dossios), and therefore was inclined to admit that flesh may contain sometimes, but not always, two different isomers of lactic acid. Wislicenus believes that the preparation of either the synthetical ethylene lactic acid, or of the second sarkolactic acid (both of which he

believes to be identical) in the pure state is so difficult a matter as to be almost impossible. He gives certain proofs of their identity, but they are mostly negative, such as the amorphous and syrupy condition of salts, and the solubility in almost absolute alcohol, and leaves it to be surmised that the malonic acid, which was formed by his pupil Dossios (*Ann. Chem.* 146, 1868, 168) under his eyes, from crude sarkolactic acid, was derived from this ethylene sarkolactic acid. But as the acid was only obtained in small quantity, and from a crude product, its use for structure formulæ is still less justified than it was for the purpose for which it has been used so many years.

The preparations of lactic acid from the brain of man and the ox, which I have described above, leave no room for doubt regarding their nature; they are specimens of the one optically active sarkolactic acid, yielding the precisely characteristic zinc salt; that they did not yield the ordinary calcium salt is of little consequence as the question of the composition of the calcium salts of sarkolactic acid is not exhaustively answered. Whatever may be the issue of the discussion regarding the constitution of the different lactic acids, *the facts* now ascertained regarding cerebral lactic acid cannot thereby be affected.

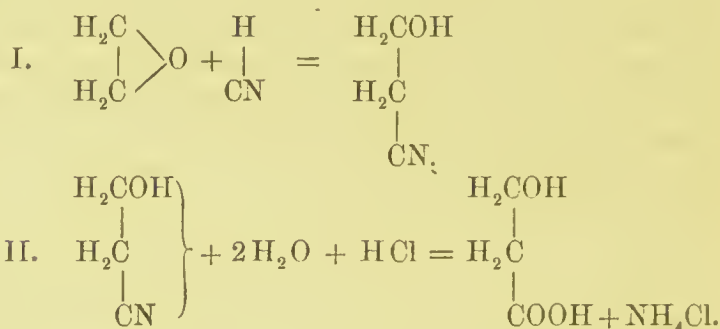
I have stated the opinion above quoted regarding the alleged second acid in sarkolactic acid already in 1877 (compare my *Pathology of the Urine*, 1877, p. 461 *et seq.*) I now prove for the brain, what Erlenmeyer has proved for the flesh, namely, that it contains only one lactic acid.

In an article in *Ann. Chem.* 191, 1878, 261, Erlenmeyer has subjected the question of ethylene lactic acid to an historical and experimental critical review. He had formerly not found ethylene lactic acid in flesh-lactic acid prepared by Liebig's method, and now endeavoured to find it by the method of Wislicenus; but he again failed, and obtained only some fermentation lactic acid. A similarly negative result has been obtained by Klimenko (*Ber. d. Ch. G.* 9, 1604). The result of this part of the review is that Wislicenus had obtained a lactic acid containing nitrogenous matters as impurity.

Erlenmeyer now produced the lactic isomer from ethylene chlorhydrine by transforming this into the nitrile  $C_3H_5NO$ , and the latter into the lactic acid isomer by two methods; one,

that of Wislicenus, namely, heating with caustic soda; another of his own, namely, heating the nitrile with fuming hydrochloric acid. Both processes gave him an acid which was shown to be identical with the hydracrylic acid of Wislicenus; for it gave, on heating with hydriodic acid in a sealed tube  $\beta$ -iodopropionic acid. This last transformation had been vainly tried by Wislicenus (*Ann. Chem.* 167, 352), and it was mainly on the basis of this failure that he declared his acid from ethylene chlorhydrate to be ethylene-lactic acid, and denied that it was hydracrylic acid.

Erlenmeyer next produced ethylene-cyanhydrine  $C_3H_5NO$  by direct combination of ethylene-oxide and prussic acid (a synthesis already unsuccessfully attempted by Wislicenus, *Ann. Chem.* 167, 346), and from this ethylene-cyanhydrine he produced the ethylene-lactic isomer, according to the following formulæ:



The reaction II. is, however, not the only one which ethylene-cyanhydrine gives on boiling with hydrochloric acid, acrylic acid,  $C_3H_4O_2$ , being formed at the same time, which can be separated by distillation, under diminished air-pressure, with the aid of fractional saturation. And this also yielded the  $\beta$ -iodopropionic acid by the process above described. The lactic isomer also behaved like the hydracrylic acid of Wislicenus (= Beilstein's hydroxyacid from iodopropionic acid) in this, that when a concentrated solution of its zinc salt was mixed with a concentrated solution of its calcium salt, the mixture yielded the double salt described by Heintz (*Ann. Chem.* 157, 291) in characteristic very little soluble crystals. (Dried in the exsiccator they are free from water of crystallisation, and contain 8.68 per cent. Ca and 14.10 per cent. Zn.)



From the foregoing it is evident (1) that the appellation of 'ethylene lactic acid' cannot be applied either to the zymo- or to the sarko-lactic acid; (2) that it is uncertain whether one or both of these isomers are ethylidene lactic acid, and whether consequently they really differ in their chemical structure; (3) that the acid termed by Heintz ethylene lactic acid is really such, and is also hydracrylic, as claimed by Wislicenus; (4) that this hydracrylic acid has not yet been shown to be present, either in the muscles or the brain of animals. The four assumed isomers of the formula  $C_3H_6O_3$  are therefore reduced to three, and the question regarding the constitution of the zymo- and sarko-lactic acid remains open.

10. *Formic Acid from the Water Extracts.*—The acids extracted by ether after precipitation of the alkaloids were in one experiment placed in a retort, and subjected to distillation. The main part of the distillate consisted necessarily of acetic acid. The acids were neutralised by barytic carbonate and evaporated to crystallisation; the first crystals gave 53.09 per cent. Ba (acetate requires 53.72 per cent. Ba); the second crystals gave 55.53 per cent. Ba, and gave a strong formiate reaction with nitrate of mercurous oxide. It is, therefore, probable, that the water extract of the brain contains a small amount of *formic acid*, as has already been stated by Von Bibra and Müller. (See *Report*, New Series, No. III. 1874, p. 206.) The amount was, perhaps, not greater than that of succinic acid to be described.

11. *Succinic Acid as a Normal Ingredient of the Brain of the Ox.*—The mother liquors of the zinc lactate gave with ferric chloride, not in the cold, but on boiling, a rust-coloured precipitate, soluble in excess of chloride, forming a dark red solution. The united mother liquors were cautiously precipitated while boiling, and the compound filtered off. They were next boiled with barytic carbonate, and the precipitate filtered off. The latter was extracted with hot dilute acetic acid, in which the ferric precipitate was insoluble. The ferric precipitates were dissolved in water, and  $H_2SO_4$ , and extracted with ether; the ether solution left the acid free.

*The acid*, easily soluble in water, crystallised on evaporation. Fused, and sublimed in white vapours. Sublimate white; no charcoal. Pungent smell of vapours. Sublimate all crystals.

In a mixture of  $\text{BaCl}_2$ ,  $\text{NH}_4\text{HO}$  and spirit it produced an immediate thick white precipitate. The sublimated crystals dissolved in water, and gave a clear and colourless solution. This was cautiously (litmus added) neutralised by sodic carbonate. The solution gave rusty precipitate with ferric chloride, soluble in excess of chloride. It gave a white precipitate with mercurous nitrate, not altered by boiling; gold chloride gave no reaction which excludes malonic acid; uranic nitrate gave no reaction. Precipitate (after boiling and concentration) with  $\text{BaCl}_2$  immediately. This was soluble in  $\text{HNO}_3$ , and reprecipitated by  $\text{NH}_4\text{HO}$ ; still more by a little spirit.  $\text{BaCl}_2$  also gave precipitate with  $\text{NH}_4\text{HO}$  and spirit, difficultly soluble in acetic acid.

This acid is consequently *succinic*,  $\text{C}_4\text{H}_6\text{O}_4$ .

12. *Succinic Acid in the Human Brain*.—The process described in the foregoing note regarding the brain of the ox was repeated on the mother liquor of zinc lactate from the brain of man, and exactly identical results were obtained. The ferric salt was decomposed and the acid extracted by ether. It remained in a crystalline state and was sublimed. The reactions were then made upon the sublimate, and found to agree exactly with those of the succinic acid.

This experiment was repeated on a second quantity with identical results.

Succinic acid is thus shown to be a normal ingredient in small quantity in the brain of man and of the ox. Müller, when extracting lactic acid from brain, had searched for succinic, but not obtained any. This is explicable on several grounds: firstly, his method was not calculated to obtain it (he waited for crystals to form in the concentrated lactic acid); and, secondly, the quantities of brain matter employed by him were probably too small. (See *Report*, New Series, No. III. for 1874, p. 232.)

The significance of succinic acid in nerve marrow is probably connected with that of the disintegration of the albuminous substances. But the possibility of merely accidental presence must not be lost sight of, as succinic acid is present in many kinds and parts of vegetables used for food of man and animals, and in wine and other fermented liquids, in which it is produced by fermentation from sugar.

## VII.

ON THE CHEMICAL DECOMPOSITION OF BILE AND  
ITS INGREDIENTS, WITH REFERENCE TO THE  
THEORY OF THE FUNCTION OF THE LIVER.  
(Summary).

*Preparation of glykocholic acid from ox-bile; Variation of the quantity of this acid in the bile from different animals.—*

This subject has given rise to some controversy, which is now in a fair way of being explained. Hüfner (*Journ. Pract. Chem.* 10, 1874, 267) had published a new mode of obtaining this acid, which was quite unsuccessful in other hands and other places than Tübingen. It was the following: To fresh bile contained in a high cylinder some ether is added, so that it floats on the surface; hydrochloric acid is now added until the bile shows a milky turbidity; crystals soon form in the liquid, under certain conditions so rapidly that after some minutes the liquid is transformed into a solid mass of crystals. As soon as the crystallisation is completed, the ether is poured off, the crystallised mass is stirred up with a large quantity of water, placed on a large filter and washed with cold water until the filtrate, green at first, has become colourless. The crystals remaining on the filter are dissolved in boiling water, the solution is filtered hot; the filtrate on cooling deposits pure glykocholic acid. The filtrate from the first crystals contains the taurocholic acid and salts.

On extending his observations, Hüfner (*Journ. Pract. Chem.* 19, 1879, 302), found that of a hundred specimens of ox-bile collected at Tübingen, forty gave this crystallisation in a few minutes, another forty gave it slower, sometimes only after the lapse of hours, and in these there was also less crystallised matter, while twenty per cent. gave no crystals at all. The inquiry was now extended to the animals from which the bile

came, and it was ascertained that the bile of bulls always gave the crystals, that of cows yielded them as a rule, with exceptions, that of oxen (castrates) never. The bile of calves never gave crystals. Whether the animals were fat or lean did not seem to exercise any influence upon the bile in respect of its crystallisability under the circumstances here considered; green bile never gave crystals, light brown bile nearly always gave them.

In respect of the causes of these variations, no reliable data have been ascertained, but it is probable that the nature of the food is one of their principal conditions. Kolbe treated many dozen specimens of bile at Leipzig after Hüfner's prescription, and never, not even with brown bile, obtained any crystals. But he observed a deficiency of sodium, as well as taurocholic acid, and is inclined to attribute the deficiency of the biliary acids to a primary deficiency of common salt in the food, and then in the blood and liver of the animals.

*Extraction of taurocholic acid and cholin from the first acid filtrate from the crystals of glykocholic acid.*—The united filtrate and washings are neutralised with sodic carbonate, evaporated to a syrupy consistency, mixed with animal charcoal, dried on the water bath, and the residue is powdered; this is now exhausted with boiling alcohol, the alcoholic solution is freed from alcohol by distillation, the residue is dissolved in water, and mixed with excess of basic lead acetate. After some standing, a lead salt settles in the shape of a glistening faintly-yellowish brown mass, lead taurocholate, from which the supernatant liquid can be easily decanted. From this precipitate taurocholate of soda is easily obtained and transformed into crystals by the addition of ether to the alcoholic solution.

The liquid decanted from the lead taurocholate is freed from lead by hydrothion; the filtrate is evaporated to suitable concentration, and on addition of platinic chloride and alcohol yields the double salt of cholin. It is better to isolate the cholin by phosphomolybdic acid, and decompose the compound with baryta before applying the platinic chloride, as without that precaution the resulting platinum salt is liable to be contaminated with potassium and volatile alkalies.

*Chemolysis of bile by baryta. Production of cholic acid.*—Tappeiner produces cholic acid by dissolving one part of



inspissated ox-bile in from ten to twenty parts of water, adding five parts of baryta water saturated boiling, and boiling the mixture during from five to seven days without interruption. The mixture is allowed to stand during a day to deposit baryum salts of fatty acids, filtered into large flasks, mixed with some ether, and then with hydrochloric acid, with the precaution of keeping most of the colouring matter in solution. After from one to three days crystals begin to appear on the surface of the precipitated cholic acid, and after from three to four weeks the entire amount of the acid is crystallised. It is filtered from the brown mother liquor, washed, and obtained pure by two crystallisations from alcohol. If the acid is deposited in hard cakes instead of loose masses of crystals, it must be triturated in a mortar and washed with small quantities of extremely dilute soda to dissolve all colouring matter, and after filtration washed with water.

If the cholic acid after addition of the hydrochloric acid is allowed to stand too long in the mixture, it is liable to become mixed with a blue ferruginous body which forms gradually in the mixture; this is soluble in alkalies with a brown colour, but again precipitated from them with a blue colour; it is amorphous, insoluble in alcohol, changed by this agent into a green colouring matter, and is difficult to collect, as it either passes through the filter or stops its pores.

*Preparation of cholic acid from pure glykocholic acid.*—Fifty grms. of glykocholic acid are mixed with 200 grms. of caustic baryta and six litres of water, and kept boiling during sixteen hours. The solution is filtered hot, but mixed with hydrochloric acid only after it has become cold. The cholic acid then falls in a sand-like state. It is washed repeatedly with warm water, and then dissolved in hot alcohol. From this it crystallises soon in octohedra and tetrahedra of the usual small dimensions. The crystals amount to 80 per cent. of the calculated quantity.

*Cholate of ethyl.*—Twenty parts of cholic acid are dissolved in 140 parts of alcohol of 90 per cent. strength, and into this solution a current of dry hydrochloric acid gas is passed. (It is necessary to prevent the mixture from getting warm, for in the case of its temperature rising during the reaction it becomes

turbid or reddish, and an uncrystallisable product is separated.) The mixture saturated with hydrochloric gas is at once, and again without allowing its temperature to rise, mixed with an equal bulk of strong alcohol. Of this mixture every 100 c.c. are allowed to flow in a thin stream into a litre of cold water. The mixture is milky at first, but after some hours shows needles, and after some days the crystallisation of the cholic ether is completed. They are washed with water, redissolved in alcohol, and reprecipitated in water, whereupon they are pure.

*Cholamide*.—Purest cholic ether is heated with highly concentrated ammonia in a sealed tube during six days to  $130^{\circ}$ , the product is diluted with nine parts of water, boiled up and filtered. On cooling silky needles of cholamide,  $C_{24}H_{41}NO_4$ , are deposited. It is very hygroscopic, easily soluble in alcohol, less easily in ether; it is little soluble in water, even on boiling, and is deposited from this in needles. The solutions have a neutral reaction. When dried at  $115^{\circ}$  in a current of hydrogen it fuses at  $130^{\circ}$ .

*Oxydation of cholic acid by means of dichromate and sulphuric acid*.—This subject has been further studied by Tappeiner (*Lieb. Ann.* 194, 1878, 211). The mixture consists of ten parts of potassic dichromate and fifteen parts of oil of vitriol, the latter diluted by three times its volume of water, or even more; and with this quantity of liquid one part of cholic acid is treated. The warm mixture is poured upon the powdered cholic acid contained in a flask; carbonic and acetic acid are immediately set free, and the rest of the cholic acid forms a fused layer on the top of the liquid; the mixture is agitated until the fused acid is again hard and granular. If a volume of more than a litre of mixture in all is treated at once, no external heat is required to effect the first stage of oxydation; this first stage consists in the formation of cholesteric, stearic, and lauric acid, besides the carbonic and acetic already mentioned; and of an acid which is not very well defined, but passes, during the second stage of the oxydation, with the aid of external heat, into an acid termed cholanic.

*Cholesteric acid*.—By this name Redtenbacher (*Lieb. Ann.* 57, 162) and after him Schlieper (*Lieb. Ann.* 58, 377) and Gundelach and Strecker (*Lieb. Ann.* 62, 205) signalled an

acid which they obtained from cholesterin, choloidic, cholic and hyocholinic acids by oxydation with nitric acid. The silver salt, which was apparently crystallised, led to the formula for the acid of  $C_8H_{10}O_5$  ( $C=6$ ); when this had to be doubled, the acid was declared to be two-basic. This acid is now shown to be a mixture of two acids, one crystalline, to which the name of cholesteric acid is continued,  $C_{12}H_{16}O_7$ , and another, the pyro-acid of the first, amorphous pyro-cholesteric acid,  $C_{11}H_{16}O_5$ . Cholesteric acid is obtained at the end of the first period of oxydation by filtering the liquid from the resin through glass wool, and evaporating at a gentle heat; it deposits in fine crystals and membranes; but when the acid is too concentrated, or the heat too great, it is transformed into the pyro-acid under effervescence. To avoid this with certainty it is useful to neutralise the greater bulk of the free sulphuric acid before evaporation. The crystallised cholesteric acid is collected by filtration through glass wool, washed with a little cold water, and dissolved in ether, from which it crystallises in needles.

*Properties.*—Much more soluble in hot water than in cold; crystallises from the hot saturated solution on cooling in small needles, which form spherical masses. From alcohol it crystallises also in needles. It is obtained in long prisms without water of crystallisation by abandoning to spontaneous evaporation its dilute watery solution covered with some ether. The crystals then form principally at the plane of contact between water and ether. The acid is not volatile with steam. Its alcoholic solution turns the plane of polarisation a little to the right, but its specific rotation is probably not more than from  $+8$  to  $+10^\circ$ .

It does not give any colour with sulphuric acid and sugar, and its sodium salt, in doses of from 0.5 to 1.5 grms. injected into the jugular vein of dogs of from 6 to 8 kilos in weight, or of rabbits, produces no toxic effects.

Theory of  $C_{12}H_{16}O_7$ :

C	.	.	52.94
H	.	.	5.88

*Salts.*—Cholesteric acid is tribasic, and yields salts of the composition  $C_{12}H_{13}O_7M^1_3$ ,  $C_{12}H_{15}O_7M^1$ , and mixtures of  $C_{12}H_{14}O_7$

$M'_2$  and  $C_{12}H_{13}O_7M'_3$ . They are all amorphous with the exception of the silver salt,  $C_{12}H_{15}O_7Ag$ . The salts with alkalies are little soluble in alcohol, so that when an alcoholic solution of the acid is mixed with an alcoholic solution of potash or soda, a syrupy precipitate is at once obtained which dries over sulphuric acid to a white amorphous hard mass. Such a salt made by potash was mainly the tribasic mixed with a little dibasic, and yielded 27.3 per cent. K instead of the 30.58 K required by the tribasic salt. The calcium and baryum salts are distinguished by their lesser solubility in hot than in cold water, their cold saturated solutions become turbid by heat, and clear up again on cooling. The acid cannot be saturated by boiling with carbonate of calcium or baryum; the quantities of base taken up remain between those required by the tri- and the dibasic salts.

At  $100^\circ$  these salts lose constantly in weight, and are transformed into salts of the pyro-acid. The tribasic saturated baryum salt is obtained by boiling the acid with baryta water, precipitating the excess of baryum by carbonic acid, filtering hot, and evaporating the solution over sulphuric acid. It is an amorphous white powder, and more stable at  $100^\circ$  than the non-saturated salts; its solution has an alkaline reaction; it is insoluble in alcohol. Its formula is  $C_{12}H_{13}O_7ba_3$ , and the amount of baryum contained in it 43.22 per cent. The silver-salt  $C_{12}H_{13}O_7Ag_3$ , is obtained by oversaturating the watery solution of the acid with ammonia and adding silver nitrate, as a white flaky precipitate, insoluble in alcohol and water, somewhat soluble in hot ammoniac nitrate, reprecipitated from this on cooling in the amorphous state. The salt bears heating to  $140^\circ$  without blackening much. This is the salt obtained by Redtenbacher and other inquirers mentioned above, as is evident from a comparison of the analytical data.

$C_{12}$	.	.	24.22
$H_{13}$	.	.	2.19
$Ag_3$	.	.	54.63

Another silver salt,  $C_{12}H_{15}O_7Ag + H_2O$  is obtained by adding to a moderately concentrated solution of the acid in water nitrate or acetate of silver; the isolated precipitate is mainly soluble in



alcohol with a strongly acid reaction, and after evaporation of the solvent remains in crystals, probably rhombohedric forms of the hexagonal system; the salt is much less soluble in water than the free acid; in the amorphous state it fuses at temperatures below  $100^{\circ}$ , but crystallised it remains unchanged at  $110^{\circ}$ .

Calculated in  $100^{\circ}$

Ag 28.49

H<sub>2</sub>O 4.7

*Transformation of cholesteric into pyro-cholesteric acid.*—

At a temperature above  $100^{\circ}$  cholesteric acid gives out carbonic acid, and is transformed into pyro-cholesteric acid, according to the equation



The transformation can be effected quickly by heating the acid to  $198^{\circ}$  in a paraffin bath; it fuses to a brownish mass with strong effervescence, and the new product is soluble in water, alcohol and ether with acid reaction; the solution decomposes sodic carbonate with effervescence; the acid is not volatile with steam, and fuses at  $108^{\circ}$ .

Theory:  $C_{11} = 57.9$  per cent.;  $H_{16} = 6.8$  per cent.

When cholesteric acid is boiled with sulphuric acid of 25 per cent. strength it is at first transformed into the pyro-acid; but later more carbonic acid is given out, and the liquid becomes dark brown. After five hours' boiling the mixture yields to ether several acids, of which one can by distillation be isolated and recognised as similar to acetic acid, another remains behind, soluble in hot water, and deposited on cooling in membranes and flakes. The latter contains 63.0 per cent. C and 6.3 per cent. H.

Cholesteric acid, when mixed with even small quantities of pyrocholesteric, loses its readiness to crystallise from water, remains on evaporation as a syrup, on drying as a gum; from ether it is crystallisable even when mixed with somewhat more pyro-acid.

*Influence of nitric upon cholesteric acid.*—Cholesteric acid dissolves easily in hot nitric acid, diluted with one-third part of water. The solution may be boiled for twenty hours, and after

the nitric acid has been distilled off, the residue in water extracted with ether. The residue from the ether solution is an uncrystallisable syrup, and seems, from its silver salts, to be a mixture of much cholesteric and little pyrocholesteric acid. Such was probably the cholesteric acid of Redtenbacher; his acid moreover contained perhaps small quantities of nitro-compounds. But the impurity was not so great as to affect the silver salt essentially.

From cholesterin, cholesteric acid cannot easily be obtained by the process applicable to cholic acid. Experiments made in that direction have hitherto failed.

*Fatty acids*.—A great number of fatty acids are produced during the oxydation of cholic acid, which form a complicated mixture. This is collected on a funnel, over glass wool, through which the liquid filters; the insoluble fatty acids are treated with dilute hot soda ley, filtered hot from the chromic oxyde, and allowed to cool. The filtrate gelatinises. The addition of dilute hydrochloric acid precipitates the fatty acids, and cholanic acid, while chromic oxyde now remains in solution. The precipitated acids are washed, and digested with dilute baryta water. The cholanate of baryum goes into solution, while the fatty acid salts remain insoluble. The latter, on decomposition by hydrochloric acid, yield the white fatty acids. The mixture of these fuses at  $53$  to  $55^{\circ}$ , which indicates a mixture of myristic and stearic acid.

*Stearic acid*, fusing at  $68.5^{\circ}$ , was isolated from the mixture by repeated fractional precipitation with barytic acetate, and identified by analysis of the acid and its baryum soap.

*Lauric acid*,  $C_{12}H_{24}O_2$ , was obtained by oxydising a mixture of fatty acids obtained like the stearic, with dichromate mixture, until the fusing point had fallen to  $43.6^{\circ}$  and did not alter any further. This acid and the acids just above are very resistant to the influence of the oxydising mixtures; yet much lauric acid must again decompose before the higher acids are oxydised down to lauric acid.

*Cholanic acid*,  $C_{20}H_{38}O_6$ .—When the mixture of fatty acids is treated by baryta water as above described, the baryta salt of this acid goes into solution. The excess of baryta is removed by carbonic acid and boiling, and the filtrate is evaporated at

a low heat until it begins to crystallise; the mixture is then rapidly heated to boiling, when a white crystalline baryum salt separates, and can be collected on a hot funnel and washed with boiling water. The concentrated mother liquors give by repeated treatment like this nearly all the cholanate in a crystalline form; the last residues of the acid can be obtained by precipitation with hydrochloric acid.

Cholanic acid is soluble in water, alcohol and ether. One hundred c.c. of absolute alcohol of  $20^{\circ}$  dissolve 2.55 grms. of the acid; hot alcohol dissolves more, and deposits the acid on cooling in very thin prisms free from water of crystallisation. Similar crystals are obtained when the acid crystallises from ether, or from the solution of one of its salts in water, to which acid and some ether have been added. The amorphous flakes at first deposited pass into the crystalline state within a few hours. Without the ether the precipitates do not become crystalline even after weeks of repose.

Cholanic acid is little soluble in cold water, but more soluble in boiling water; from this it is deposited on cooling in fine needles which, after drying on the filter, form a shining felt. One part of acid requires for solution 4000 parts of boiling and 9174 of water of  $20^{\circ}$ .

The acid crystallised from alcohol may be heated to  $250^{\circ}$  without change, but at higher temperatures it fuses and is decomposed.

The alcoholic solution turns the ray of polarised light to the right. Specific rotation about  $+53^{\circ}$ . The acid does not give Pettenkofer's reaction. It dissolves in oil of vitriol, the solution fluoresces similar to that of cholic acid; on addition of water the acid crystallises on cooling, apparently unchanged.

1 gram. of its sodium salt, injected into the jugular vein of dogs (5 to 8 kilos) produces no symptoms. Two grms. introduced into the stomach act as a cathartic. Cholanic acid forms with bases two series of salts,  $C_{20}H_{27}O_6M^1$ , and  $C_{40}H_{51}O_{12}M^1_5$ ; the latter are probably double salts of the components  $C_{20}H_{26}O_6M_2 + C_{20}H_{25}O_6M^1_3$ .

*Potassium salt*,  $C_{40}H_{51}O_{12}K_5 + 6H_2O$ , is obtained by boiling the alcoholic solution of the acid with potassic carbonate during at least twelve hours. The alcoholic solution is evaporated to

dryness and extracted with absolute alcohol. On addition of ether to this solution a turbidity ensues, and after some time the salt is crystallised. It is soluble in water and very deliquescent in air.

$$\text{H}_2\text{O} = 11.7 \text{ per cent.}$$

$$\text{K} = 21.24 \quad ,,$$

There are other salts containing from 9.9 to 13.4, and 15.0 per cent. K. They are not yet well defined.

*Baryum salts*;  $\text{C}_{43}\text{H}_{51}\text{O}_{12}\text{ba}_5 + 5 \text{H}_2\text{O}$ .—This is the salt obtained in the first separation of the acid after removal of the fatty acids, by boiling.

Theory:	C	.	.	45.05
	H	.	.	4.78
	Ba	.	.	32.14
	H <sub>2</sub> O.	.	.	8.4

The same salt crystallises from *cold* water with 7 H<sub>2</sub>O, or 11.6 per cent., in white crusts of felted fine needles.

$\text{C}_{20}\text{H}_{27}\text{O}_6\text{ba} + \text{H}_2\text{O}$ . When the solution of the salt with 5 baryum equivalents is treated with carbonic acid to complete saturation with this gas, the salt of the foregoing composition is gradually deposited in fine needles, without any barytic carbonate being mixed with it. The salt is little soluble in cold water, more in hot, and crystallises from this solution on cooling in needles. It is insoluble in alcohol.

Theory:	C	.	.	55.61
	H	.	.	6.25
	ba	.	.	15.87
	H <sub>2</sub> O.	.	.	4.1

The lead salt,  $\text{C}_{40}\text{H}_{51}\text{O}_{12}\text{pb}_5$ , is precipitated from an alcoholic solution of cholanic acid by lead acetate; white amorphous powder, very little soluble in even hot alcohol. Contains 41.56 per cent. of Pb.

The silver salt,  $\text{C}_{40}\text{H}_{51}\text{O}_{12}\text{Ag}_5$  is obtained by adding to the baryum salt of analogous composition silver nitrate, as a white curdy precipitate, very little soluble in water or alcohol, soon blackening on exposure to light.



Theory :	C	.	.	38.0
	H	.	.	4.0
	Ag	.	.	42.75

Cholanate of ethyle,  $C_{20}H_{27}O_6 \cdot C_2H_5$ , is formed by passing hydrochloric acid gas into a solution of the acid in alcohol. Is precipitated by water after 24 hours, and washed with dilute soda, recrystallised from alcohol.

*Bearing with hydrochloric and with nitric acid.*—Cholanic acid can be boiled with a mixture of hydrochloric acid and water in equal parts without undergoing much change. But a hot mixture of equal volumes of nitric acid and water dissolves cholanic acid rapidly under evolution of nitrous acid. When the action is completed long hair-fine crystals are deposited which are very little soluble in cold, slightly more soluble in hot water. They resemble choloidanic acid, first obtained by Theyer and Schlosser (*Ann. Chem.* 50, 243) and later by Redtenbacher (*Ann. Chem.* 57, 145)  $C_{16}H_{14}O_7$ .

Theory :	$C_{16}$	.	.	58.53
	$H_{24}$	.	.	7.31

*Oxydation with permanganate* in the cold of a dilute solution of the acid gives oxalic acid, and traces of butyric, and several other products, of which the most abundant is an acid of the formula  $C_{24}H_{36}O_{15}$ . This acid dried *in vacuo*, is a glass-like brittle mass, very soluble in water and alcohol, little soluble in ether; dissolved in absolute alcohol and treated with a stream of hydrochloric acid gas, it forms an ether.

*Treatment of cholic acid with reducing agents at high temperatures* (A. Destrem, *Compt. rend.* 87, 1878, 880).—The acid was distilled with zinc-powder and gave a hydrocarbon corresponding to the formula  $C_{24}H_{32}$ ; it begins to distil at  $215^\circ$ , and the temperature rises to  $325^\circ$ ; the last portions which distil over are very viscous; crystalline needles are deposited in the neck of the retort.

*Theory of the oxydation of cholic acid.*—As in this process cholesteric acid is formed before stearic and cholanic acid, it cannot be formed from either of them: moreover neither gives by oxydation cholesteric acid; further, cholanic acid cannot be

formed from stearic. The three bodies can therefore be derived from the molecule of cholic acid as ordinarily written,  $C_{24}H_{40}O_5$ , only on the supposition, that the process of cleavage takes place in different molecules of the same acid according to different modes; it is even necessary to assume that some molecules take up hydrogen; neither of these assumptions could claim attention in view of the probability that stearic acid is not the highest fatty acid formed in the process, and that already at the beginning of the oxydation considerable quantities of acetic and carbonic acid are set free. The bodies obtained can be derived from cholic acid on the supposition either that the molecule is in reality two or three times as great as is at present assumed, or that during the progress of oxydation fragments of  $C_{24}H_{40}O_5$  combine, and form products of condensation so called. The latter hypothesis has not much in its favour, and the circumstance that nitric acid gives products analogous to those obtained by chromate mixture is particularly against it. The acetic, butyric, caprylic and caprinic acid formed cannot be derived from choloidanic or cholesteric acid, for choloidanic acid is not changed by nitric acid, and cholesteric acid is only changed slowly into pyrocholesteric acid.

The oxydation products of cholic acid show that the formation of the biliary acids in the liver has probably some connection with the economy of the liver as regards fats. Tappeiner does not think it probable that the biliary acids are produced in the liver synthetically, but accepts them as derivatives of albumen, and claims thus to have produced stearic and lauric acid from albuminous matter.

The coincidence of increased formation of bile and accumulation of fat in the liver after every meal is probably not accidental but relational. The cohesion of glycogen with these processes Tappeiner does not even allude to. The hypothesis that there may be several cholic acids, all of the formula  $C_{24}H_{40}O_5$ , and yet differently constituted, isomers, not separable by ordinary means, is one of the few possible ways out of what is at present a very complicated position.

## VIII.

*CHITIN, THE SIMPLEST NITROGENISED ORGANO-  
PLASTIC AMYLONIDE. (Summary and Additions.)*

CHITIN was first distinguished from horn substance by Odier in 1823, and supposed to be free from nitrogen (*Mém. Soc. d'Hist. Nat. de Paris*, 1, 29). Lassaigne recognised that it (named by him entomaderm) contained nitrogen (*Compt. rend.* 16, 1087; *Journ. Chim. Méd.* 19, 379). It was next studied by Payen (*Compt. rend.* 17, 227), and more intimately by K. Schmidt (*Ann. Pharm.* 54, 298). Schlossberger (*Ann. Chem.* 98, 105) contributed to its more accurate knowledge by defining the organic matter contained in some shells, and in the byssus of shells which had been confounded with chitin, as conchiolin. Berthelot (*Compt. rend.* 47, 227; and in detail *N. Ann. Chim. Phys.* 56, 149) found that chitin on chemolysis with acid gave a kind of sugar. Peligot (*Compt. rend.* 47, 1034; *N. Ann. Chim. Phys.* 58, 83), in consequence, endeavoured to find the amylumlike body in chitin which gave this sugar. Städeler (*Ann. Pharm.* 111, 21) also studied chitin, and gave a new formula differing from that first given by Schmidt. G. Ledderhose (*Zeitschr. Physiol. Chem.* 2, 1878, 213) found the sugarlike body to contain nitrogen, and termed it glycosamin. He also proposed a new formula for chitin. Frémy's and Rouget's chitin, see below, pp. 138 and 139.

*Occurrence.*—Chitin forms the principal constituent of the dermatic envelope and entire firm skeleton, tendonlike parts, and attachments of muscles of nearly all articulate animals, and of insects not only in the fully developed, but also in the larval and pupal state. It is also said to be present in the lining membranes of the tracheæ of air-breathing articulates, and forms part of the gills of those breathing water; it is also a constituent of the lining membrane of the intestinal canal. It

possesses great flexibility, when it forms the external organs, membranes and hairs of animals by itself, combined only with little inorganic salt and organic colouring matter; but it acquires great hardness by an impregnation, in the course of its structural development, with carbonate and phosphate of lime, and then shows as dermatic skeleton analogy with the ossein of the skeleton of the vertebrate animals.

As the external envelope of animals of the class of articulates is formed gradually, and is changed repeatedly during their growth and life, it is advisable to bear in mind that the chitin obtainable by chemical proceedings may not be the same in the same species at all times, and may differ in different species. The bones of young vertebrates yield chondrin, those of adult ones ossein; the growing antlers of the stag yield chondrin only, when just fullgrown they yield chondrin and ossein, and after maturation and scouring ossein only.

*Modes of preparation.*—The materials which yield chitin with the least trouble are perhaps, in places near the seashore the transparent crustaceans, such as shrimps, prawns, and langostinos; but in inland countries the wing-cases of coleoptera. If entire insects are taken it is necessary to remove mechanically the internal organs, particularly the intestinal canal, to exclude the possible admixture with the chitin of any cellulose from leaves or other vegetable structures which have served as food. The shells of larger aquatic crustaceans are also a convenient and easily procurable material, although they require prolonged treatment with much acid for the removal of the calcic carbonate and phosphate.

The materials selected are exhausted by boiling with caustic soda, prolonged treatment with dilute hydrochloric acid, second boiling with caustic soda, then with water, and long boiling with alcohol in succession. If the chalky shells of large articulates are used the following succession of operations may be adopted. The shells are pounded in a metal mortar, until all tubes are crushed, and all stiff parts comminuted. The pasty mass is now boiled with strong caustic alkaline ley, and the latter is repeatedly changed; when all matter soluble in alkali is removed, the well-washed mass is placed in dilute hydrochloric acid, and treated with this agent until no further effervescence



is perceived on standing, and until all particles are soft and translucent. The residue is now washed, and boiled with spirit until all colouring and other soluble matter is extracted. The chitin thus obtained is dried on the water-bath, and shrinks to a tough, shrivelled, greyish white mass of particles. These may be ground in a coffee-mill. Chitin thus prepared can be purified, according to Städeler, by boiling it for 12 hours or longer with a mixture of 1 vol. of oil of vitriol and 4 vols. of water, when a part of it is changed into soluble matters, crustacin (alkaloid), and chitose (sugar), while another part remains as a swelled pasty mass, being almost pure chitin free from ash. This may be washed by decantation as long as it contains free acid, but as soon as all the acid is removed it remains partly suspended in the liquid; the latter must therefore be evaporated to dryness.

When Peligot treated the skins of silkworms with caustic ley, alcohol, ether, and acetic acid, he obtained translucent bags, open at both ends, consisting of a chitin which contained 48.13 per cent. C, 6.90 per cent. H, 8.30 per cent. N, and 36.67 per cent. O. After this had been digested with dilute permanganate of potash, it contained 47.38 per cent. C, 7.02 per cent. H, 6.15 per cent. N, and 39.45 per cent. O. When this was treated with sulphuric acid containing 6 molecules of water, 44 per cent. of its weight was transformed into soluble products, while the residue still contained 5.8 per cent. of nitrogen. The latter was then treated for several hours with hot, or for some days with cold concentrated watery solution of potassic permanganate, then with potassic bisulphite, and finally washed with water. The remaining white mass did not evolve ammonia when boiled with potash ley. But when it was made up into a paste with potash hydrate and water, and heated to 100° for eight days it dissolved, evolving ammonia on the one hand, and forming fatty acids on the other, which were set free by acids. Even after two-thirds of the chitin had thus been decomposed, the remainder recovered from the alkali still contained 6.2 per cent. of nitrogen, and continued to evolve ammonia on renewed application of potash. When this chitin, after having been softened in water, was treated with oil of vitriol and iodine, it exhibited under the microscope brown

spots and blue particles of irregular shape. The blue colour became more plainly visible, if the skin was left for some days in the iodine solution, and then moistened with oil of vitriol whereupon entire pieces of the membrane became greenish at first, but changed to indigo blue as the iodine evaporated. From this chitin moreover, watery cuprammonia extracted cellulose, which was precipitated from the solution by acids. The horny substance of the lobster also exhibited the reaction of cellulose with oil of vitriol and iodine. Städeler declared this chitin to have been impure, as he himself could extract nothing from pure chitin by cuprammonia. But it must be borne in mind that this impurity could only have arisen from a structural admixture of cellulose with chitin, and therefore chitin which gives this reaction is unsuitable for experiments on pure chitin, as at present chitin and cellulose cannot be separated by the means ordinarily applied for the purification of chitin.

*Properties.*—Chitin, when purified by boiling with dilute sulphuric acid, or by solution in concentrated hydrochloric acid and precipitation with water, is a white amorphous mass, sometimes tough and difficult to reduce to a powder.

*Composition.*—Its elementary composition has been estimated by many observers with the following results.

	C	H	N	O
Children and Daniell <sup>1</sup> .	46.08	5.96	10.29	37.67
K. Schmidt (mean of 15 combustions) .	46.66	6.60	6.53	40.21
K. Schmidt's maxima .	46.80	6.77	6.79	—
minima .	46.48	6.43	6.32	—
Städeler . . . . .	46.32	6.40	6.14	41.14
Lehmann (mean) <sup>2</sup> .	46.734	6.594	6.493	40.179
Ledderhose (mean of 12 combustions) .	45.69	6.42	7.00	—
Ledderhose (mean of analyses with more O)	46.026	6.256	7.00	—

K. Schmidt had calculated the empirical formula  $C_{17}H_{28}N_2O_{11}$  from his analyses, having deducted from 0.6 to 2.0 per cent. of

<sup>1</sup> Todd's *Cyclopædia of Anat. and Physiol.* 2, 282.

<sup>2</sup> *Lehrb.* 1853, i. 382.

ash (chitin from crustacea). Schmidt's formula was  $C_{17}H_{14}NO_{11}$ , when  $C=6$ ;  $O=8$ ; on account of its decomposition by heat and acids, he was of opinion that chitin contained the elements of the primitive fibres of the muscles of arthropodes ( $C_3H_6NO_3$ ) with a carbohydrate ( $C_9H_8O_8$ ). But Städeler calculated the formula  $C_9H_{15}NO_6$ , which being more simple and apparently supported by the decomposition of chitin into a glucose and a hypothetical amido-acid was more generally adopted. Its theory agreed with Städeler's results as follows:

			Städeler
9 C .	. 108	46.35	46.32
15 H .	. 15	6.44	6.40
N .	. 14	6.01	6.14
6 O .	. 96	41.20	41.14
<hr/>			
	233	100.00	

The nitrogen had been found much higher by Children and Daniell, namely, 10.29 per cent.; Payen found 8.99 per cent.; Schlossberger 6.4 per cent. N. Bütschli and Emmerling (Du Bois and Reichert's *Archiv*, 1874, 362), estimated the nitrogen in chitin by the two rival methods of analysis. Bütschli found by combustion with soda lime as the mean of three experiments 6.32 per cent., by combustion with cupric oxide in carbonic acid atmosphere as the mean of two experiments 7.385 per cent. N. Emmerling (*loc. cit.* p. 370), analysed chitin which had been precipitated from hydrochloric acid solution by water and obtained by combustion with soda lime 6.24 per cent., by combustion with cupric oxide 7.02 per cent. N. In the last analysis a loss is noted to have been incurred. Ledderhose estimated the nitrogen in chitin twice by combustion with soda lime, observing the precautions otherwise known, but lately again pointed out by Makris (*Ann. Chem.* 184, 371), and obtained 6.96 per cent. and 7.049 per cent., or mean 7.00 per cent. nitrogen.

With the aid of these later data concerning the percentage of nitrogen, Ledderhose came to the formula  $C_8H_{13}NO_5$ , which requires

C	.	.	47.29
H	.	.	6.40
N	.	.	6.896

but thinking that the amount of carbon required by it was too high, as compared with that found by analysis, he preferred  $C_{15}H_{26}N_2O_{10}$ , which requires

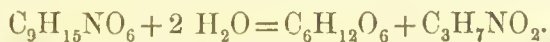
			Mean of all his analyses
C	.	45.685	45.69
H	.	6.598	6.42
N	.	7.10	7.00

But in the endeavour to support this formula by an equation intended to express the structure of the body, he unfortunately fell into an error, whereby  $C_{15}H_{26}$  in the left half of the equation are supposed to be counterbalanced in the right half by  $C_{15}H_{38}$ . The error is not a misprint, but is stated in words printed in emphatic type (*loc. cit.* p. 224 to 225), and the supposed equation is repeated twice over. That chitin was free from sulphur and phosphorus was expressly proved by Schmidt.

*Decompositions.*—When heated in the open chitin does not change in form, but simply chars, without evolving carbonate of ammonia. But when subjected to thermolysis in a closed apparatus it gives out water, acetic acid, ammoniac acetate, and a small quantity of an empyreumatic oil, while a charcoal of the original form of the chitin remains. Under the influence of air and water it is only slightly altered even after long periods; thus Schlossberger kept some chitin under water for a year, and found it only partly dissolved, the other part changed into a slimy mass. Long boiling with water does not alter the purified skins of silkworms, but the water takes up a small quantity of a nitrogenous substance, which when the solution is evaporated to a small bulk, is precipitated by tannic acid, chlorine or alcohol. Owing to this reaction Lassaigne believed that some gelatin had been formed from the skins; but this has probably no relation to the chitin itself, but was only the effect of the adhesion to the skin of, or penetration of its structure by a collagenetic matter not yet isolated. When Schmidt heated chitin with water in a sealed tube to  $280^{\circ}$ , he



observed it to become brown and brittle, but not to alter otherwise. Chitin dissolves readily in oil of vitriol, forming a thick solution; this gives a copious precipitate when immediately or after 12 hours thrown into water. The solution in oil of vitriol is colourless at first; on standing it deposits a few dark flakes; I have not observed it to become black after 12 hours, but Schmidt saw it become black after 48 hours, and perceived that it had deposited a small quantity of insoluble matter, had a choking smell, and contained acetic acid and an ammonium salt. The colourless solution of chitin in oil of vitriol may, after standing some hours, be diluted and then yields a precipitate of unchanged chitin, but if it has been heated and decomposed it yields, neutralised with barytic carbonate, a soluble baryum salt (acetate), and reduces alkaline copper solution (chitose). Berthelot dissolved chitin in oil of vitriol and dropped the solution into a hundred times its weight of water; he then boiled for an hour, and neutralised with chalk; on evaporation he obtained a sugar, which was fermentable (alcohol was isolated), and reduced alkaline copper solution. Städeler now gave to this experiment a more precise explanation. He boiled chitin with a mixture of one volume of oil of vitriol and four volumes of water for 12 hours or longer, separated what remained insoluble, supersaturated with lime (whereupon ammonia was evolved), and evaporated the filtrate to a syrup. This remained amorphous, and reduced cupro-potassic tartrate. No tyrosin, leucin, or glykokoll could be obtained from the syrup, but it contained a small quantity of an amorphous matter, the nature of which he failed to ascertain. He formulated the splitting up of chitin as follows, overlooking entirely the formation of acetic acid first found by Schmidt,



Sericin is  $\text{C}_3\text{H}_7\text{NO}_3$ . Chitin is soluble in cold concentrated hydrochloric acid without change of colour. The skins of insects when immersed in hydrochloric acid containing six molecules of water, become transparent, are disintegrated, and after a few moments dissolved. The shells of crustaceans are never entirely soluble in hydrochloric acid in the cold. After neutralisation of the solution by alkalis, tannic acid throws

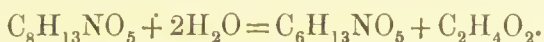
down a precipitate containing nitrogen (crustacin?). The solution of chitin in concentrated hydrochloric acid is not changed quickly, but deposits the entire amount of the chitin which it contains by suitable dilution with water. When the hydrochloric solution is mixed with alcohol two bodies free from nitrogen are obtained, the nature of which has not been ascertained by their discoverer Bütschli. One part of purified lobster shell dissolves in twelve parts of hydrochloric acid at 40°, and is immediately chemolysed. A cold hydrochloric acid solution on being boiled for some time becomes blackish-brown, and after an hour's boiling the chitin is completely chemolysed. If it is now evaporated on the water-bath, a compound crystallises in large quantities, which Ledderhose has described as hydrochlorate of glycosamin, and to which he ascribes the formula  $\text{COH}(\text{CH}.\text{OH})_4.\text{CH}_2.\text{NH}_2 + \text{HCl}$ , or  $\text{C}_6\text{H}_{13}\text{NO}_5 + \text{HCl}$ , being that of a carbohydrate in which one group OH is substituted by  $\text{NH}_2$ , while a molecule of HCl is in so-called molecular combination. The crystals are mixed with a black humuslike matter, which is completely insoluble in water, and can be separated from the crystals after expulsion of all free hydrochloric acid, by repeated crystallisation of the salt from water.

The quantity of the new salt containing the new base (which as an amide of a previously known body, chitose, we term chitosamin) obtainable from a given weight of chitin was estimated as follows. A weighed portion of chitin dried at 110° was boiled during an hour with concentrated hydrochloric acid, and evaporated to dryness; the residue was dissolved in water, and digested for some time on the water-bath, and freed from black flakes by filtration; the solution was again evaporated, dried at 100° and weighed. The product amounted to 91 per cent. of the chitin employed. This salt lost but little in weight by a repetition of the purifying process. The amount of chitosamin formed from chitin may therefore be calculated, deducting the hydrochloric acid and allowing for some impurity, to be from 70 to 75 per cent. of the chitin employed. The solution contains no ammoniac chloride, so that it seems as if the entire amount of the nitrogen contained in chitin was transferred into the chitosamin. No oxalic acid is formed, and it seems as if chitosamin was the only fixed product of the chemolysis.

The solution of chitin in concentrated hydrochloric acid is subjected to distillation to collect the volatile products. The distillation is repeated after addition of water to the liquid in the retort. The distillates are neutralised with silver oxide, the dissolved silver is removed by hydrothion, and the acid filtrate is neutralised with barytic carbonate and evaporated. A crystalline salt is obtained having the character of acetate. But there is a small amount of a higher fatty acid present, which depresses the baryta a little, as butyroacetic probably, and can be recognised by its peculiar smell.

By a comparison of the results of Berthelot with his own, Ledderhose comes to the result, that chitin by treatment with concentrated sulphuric acid, is chemolysed in the same manner as by concentrated hydrochloric acid, giving chitosamin and acetic acid; but when these products are boiled long in very dilute solution, the chitosamin is further split up into fermentable sugar and ammonia.

The decomposition of chitin under the influence of acids and heat might therefore find its simplest expression in the formula



The small amount of butyric acid obtained seems to indicate that with the simplest chitin, aceto-chitin, there occurs mixed a butyro-chitin, which contains the radicle butyryl in the place where the simplest chitin contains the radicle acetyl.

*Chemolysis of chitin with sulphuric acid.*—Twenty grms. dry pure chitin were heated in a sealed lead tube with 360 c.c. of sulphuric acid of 10 per cent. strength for 48 hours. The liquid was then found clear and to contain a reducing body and acetic acid, but a large proportion of the chitin was only softened, of the consistence of cheese, another flaky. The liquid was filtered off and the solid residue treated with oil of vitriol.

It dissolved, forming a transparent thick fluid. This with water gave a copious white precipitate of pure chitin. All the portions of liquid and precipitate were boiled for eight hours in platinum still, and three litres of distillate were drawn one after the other, water being added after each litre drawn off. The

liquid in the still contained yet much undissolved and apparently undecomposed chitin. The sulphuric acid solution gave a precipitate with phosphotungstic acid. The undecomposed chitin was treated with fresh oil of vitriol and the solution treated with water. Pure white chitin was precipitated, which after washing with water and absolute alcohol, appeared as a white amorphous mass, like starch. The solution contained an alkaloid, crustacin, precipitable by phosphomolybdic acid, and a sugar, chitose.

Chitin is coloured orange yellow to dark brown red by iodine water; addition of oil of vitriol to this test causes the chitin to dissolve, but does not produce either a violet or a blue colouration. Concentrated nitric acid dissolves chitin without discolouration; one part of chitin dissolves in an equal weight of cold nitric acid containing four molecules of water. By the addition of a large amount of water chitin is again precipitated; but some acetic acid is always formed, the more the longer the action of the acid has continued. Payen observed that crabs' claws immersed in nitric acid became transparent after a few moments; when the acid was drained off, the membrane retained at first its original form, but afterwards deliquesced to a colourless liquid, beginning at the edges.

Alkalies in the dilute or even pretty concentrated state alter or affect chitin so little, that they can be employed for its purification. The alkali may be as strong as one part of potash hydrate in three parts of water, and the chitin may be boiled in this for days, or even heated with it to  $210^{\circ}$  in sealed tubes without being sensibly attacked. But when it is fused with solid hydrate of potash, it evolves ammonia (Payen) and hydrogen (Ledderhose) without charring, and leaves a residue soluble in water. If the fusion is not continued until this mass is white, but interrupted, and the fused mass is dissolved in water, mixed with sulphuric acid, and distilled, acetic and a little butyric acid are obtained, and recognised by their baryum salt.

*Frémy's chitin, free from nitrogen.* (*Compt. rend.* 39, 1052; *N. Ann. Chim. Phys.* 43, 1855, 93).—The skeletons of crustacea are treated with cold dilute hydrochloric acid, the undissolved residue is boiled for several hours with potash-ley



and then washed with water, alcohol and ether. A transparent horny substance is thus obtained, containing on the average 43.35 per cent. C, 6.65 H, and 50.00 per cent. O, but no nitrogen, and therefore isomeric with cellulose ( $C_6H_{10}O_5$ ). This substance resists the influence of dilute acids and alkalies; acids in particular do not convert it into sugar, and fuming nitric acid has no action on it whatever. By other concentrated mineral acids it is broken up, dissolved and converted into an acid, which Frémy compares with his metapectic acid; by boiling nitric acid he obtained oxalic acid.

*Rouget's chitin and modified chitin.* (*Compt. rend.* 48, 793.)—The dermatic envelopes of the crustacea, in their natural state, or dissolved in hydrochloric acid, when treated with mercuric nitrate give a rose-red colouration; when treated with nitric acid and ammonia an orange-red colouration, and when mixed with iodo-chloride of zinc a violet reaction. (Zinc chloride solution sp. gr. 1.8 at 15° 100 parts; potassic iodide 6 parts to be dissolved in the syrup, and then as much iodine as the mixture will take up.) The skins, after ebullition with potash ley of 40°, which does not alter their appearance or structure, react more quickly with the iodo-chloride of zinc, producing a more intense bluish violet colour; but they remain still unchanged by concentrated potash ley, acetic or tartaric acid. Boiled for half an hour with five times their weight of potassic hydrate and such a quantity of water that the mixture would immediately solidify if allowed to cool, a large quantity of ammonia is liberated and half the chitin is dissolved. The residue (modified chitin) shows traces of the microscopic structure of the original tissue, is translucent, gelatinous and easily broken up while moist; in the dry state it is greyish white, dull of appearance, and light in weight. With tincture of iodine, or with iodine and dilute acetic acid, this residue becomes violet throughout; iodo-chloride of zinc colours it pure blue. It dissolves almost instantly in acetic or tartaric acid, or in warm water containing 0.5 per cent. of hydrochloric or nitric acid, and is precipitated from these solutions by alcohol or by caustic ley as a somewhat translucent paste, which dries up to a yellowish translucent gum. The acid solution and the precipitate are coloured reddish violet by a few drops of a solution of

iodine in potassic iodide. It dissolves in oil of vitriol with yellow or brown colour and is precipitated by water from the freshly prepared solution as a white powder. The solution is less completely precipitated by water after having been allowed to stand for 12 to 24 hours, and is then found to contain sugar. After being precipitated by alcohol or alkalies from its solution in acid, it is still soluble in acids, and contains nitrogen.

Purified chitin is insoluble in water, aqueous ammonia, acetic acid, alcohol and cuprammonia.

The dark amorphous humus-like substances obtained during chemolysis of chitin remain to be investigated.

*Chitosamin*,  $C_6H_{13}NO_5$ , is obtained from the sulphate by treatment with barytic hydrate without employment of heat. It crystallises from alcohol in needles.

The nitrate and sulphate are obtained by treating the hydrochlorate with nitrate or sulphate of silver; both salts crystallise in fine needles.

A double salt of chitosamin and lead chloride crystallises from water.

The hydrochlorate is obtained directly from chitin in the manner above described. It turns the ray of polarised light to the right.

The limited, or so-called specific rotation according to Lædderhose is  $+70^\circ$ , according to observation made by me in the Pathological Institute  $+72^\circ 15'$ . The rotation is more considerable immediately after solution, and falls gradually during 24 hours, after which it becomes stable at the point related.

A hundred parts of saturated solution of chitosamin hydrochlorate contain 21.83 per cent. of salt dry at 100.

All compounds reduce potassio-cupric tartrate, but do not ferment with yeast.

*Behaviour of chitin in various species of animals.*—As to organs see introduction to this chapter above, also K. Schmidt, *Zur vergl. Physiol. der wirbellosen Thiere*, Braunschweig, 1845; Frey and Leuckart, *Wagner's Zootomie*, 2, 133 and 167; Grube, *Wiegmann's Archiv*, 1853, 104; Leydig, *Müller's Arch.* 1855, 376.

On other (problematical) statements concerning the occur-

rence of tissues containing chitin in other nonvertebrates compare Schlossberger, *Vers. etc. Thierchemie*, 1856, 1, 1, 230. Leyer and Köller (*Ann. Chem.* 83, 336) and Schlossberger (*loc. cit.* 228) when chemolysing entire wing-cases of cockchafers obtained leucin and tyrosin. This therefore was due to a constituent other than chitin. Schmidt examined chitin from *Melolontha vulgaris*, *Atuchus sacer*, *Astacus fluviatilis*, *Astacus marinus*, *Squilla mantis*, and found the elementary composition as stated above. Schlossberger (*loc. cit.* 227) examined the shell of *Palinurus*, and found in its chitin 6·4 per cent. N.

The shells of crustacea contain up to 63 per cent. of organic matter; the mineral ingredients are mainly carbonate and phosphate of calcium.

Thorax of crayfish contained: organic matter 33·3 per cent.; calcic carbonate with little sodic chloride, iron, manganese, and pigment 61·0 per cent.; calcic phosphate 5·7 per cent. (John, *Chem. Schriften*, 3, 49.)

Crayfish, shells: organic matter 28·0 per cent.; calcic carbonate 60·0 per cent.; calcic phosphate 12·0 per cent. (Mérat and Guillot, *Ann. Chim.* 34, 71.)

Crayfish, thorax: chitin 46·73 per cent.; ash 53·27 per cent. In this 86·83 per cent. calcic carbonate, and 13·17 per cent. calcic phosphate. (K. Schmidt, *loc. cit.*)

Shell of crayfish: organic matter 36·5 per cent.; calcic carbonate 56·8 per cent.; calcic phosphate 6·7 per cent. (Frémy, *loc. cit.*)

Shell of crab: organic matter and water 28·6 per cent.; sodium salts 1·6 per cent.; calcic carbonate 62·8 per cent.; calcic phosphate 6·0 per cent.; magnesian phosphate 1·0 per cent. (Goebel, *Schweigger's Journ.* 39, 440.) The same inquirers found in the claws of crab: organic matter 17·18 per cent.; calcic carbonate 68·36 per cent.; calcic phosphate 14·06 per cent.; in the teeth of the claws: organic matter 12·75 per cent.; calcic carbonate 68·25 per cent.; calcic phosphate 18·75 per cent.; shell of crab: organic matter 28·6 per cent.; calcic carbonate 62·80 per cent.; calcic phosphate 6·0 per cent. (Chevreul, *Ann. gén. des. sc. phys.* 4, 124; *Schweigg. Journ.* 2, 495.)

Shells of squilla mantis: chitin 62·84 per cent.; salts 37·17 per cent. (Of these calcic phosphate 47·52 per cent., and calcic carbonate 52·48 per cent. K. Schmidt, *loc. cit.*)

Claws of lobster: chitin 22·94 per cent. In the ash of these claws 87·94 calcic carb. and 12·06 calcic phosphate. (Schmidt. *loc. cit.*)

Claws of lobster: organic matter and water 44·76 per cent.; sodium salts 1·5 per cent.; calcic carbonate 49·26 per cent.; calcic phosphate 3·22 per cent.; magnesian phosphate 1·26 per cent. (Chevreul, *loc. cit.*)

Claws of lobster: organic matter 28·0; calcic carbonate 40 per cent.; calcic phosphate 14·0 per cent.; (Guillot, *loc. cit.*)

Shell of lobster: organic matter 44·3 per cent.; calcic carbonate 49·0 per cent.; calcic phosphate 6·7 per cent. (Frémy, *loc. cit.*)

Considering that the dermatic envelope and motor parts in connection therewith, as well as respiring and digesting surfaces of three entire classes of invertebrate animals, are to a large extent made up of chitin, the foregoing data, which include all that we have been able to collect from readily accessible sources, must be considered very scanty. It is therefore to be hoped that students of comparative biology will give some attention to this interesting subject.



## IX.

ON HEMISYMMETRY IN THE CHEMICAL CONSTITUTION  
OF ORGANOPLASTIC SUBSTANCES.—HEMIPROTEIN  
AND HEMIALBUMIN. (*Summary and Additions.*)

THE experiments of chemists who operated on the albuminous substances with acids were mostly directed to effect a complete splitting up of the molecule into the most simple final products. The intermediate products of the processes were, as a rule, not studied, and with a view to avoiding their formation, or making their existence as transitional as possible, the acids were chosen of such concentration as would effect the desired purpose in the shortest time. From this practice Schützenberger deviated in one of his earlier experiments, when he subjected albumin to the influence of boiling dilute sulphuric acid for a short time, and examined the products (*Bullet. Soc. Chim. Paris*. N.S. 23, 161). He took a quantity of coagulated moist egg-albumin corresponding to 1 kilogramme of dry albumin, distributed it in 6–8 litres of water, and added 200 grms. of oil of vitriol of 66° B. The mixture was then heated during from one and a half to two hours by steam. The liquid was kept on a fixed level to avoid the formation of brown products by drying on the margins. The albumin became disintegrated and formed at last a homogeneous white gruel. To effect a more even influence of the acid the boiling may be interrupted when the albumin has fallen to small pieces, and the mixture may be worked with a brush through a fine-meshed tammy. After this operation the boiling is resumed and continued to the end of the two hours. The mixture is allowed to cool, and filtered through a bag of felt. There remains a precipitate which has great resemblance to freshly precipitated silica or

alumina, and a liquid containing the acid and its products in solution passes through. The insoluble precipitate on the filter is termed *hemiprotein*, the soluble matter remaining in the acid solution *hemialbumin*.

*Hemiprotein*.—It is washed with water until the filtrates cease to show an acid reaction. It is then white, tasteless, and has a very feeble acid reaction; it dries to a friable mass, which breaks up spontaneously into small, irregular, polyhedric, transparent, yellowish fragments, and when ground up in a mortar, yields a nearly white, non-hygroscopic powder, which is insoluble in water, alcohol and ether. The weight of the perfectly dry substance amounts to about one-half of that of the dry coagulated albumin employed. The albumin is therefore split up by a short influence of hot dilute sulphuric acid into two nearly equal halves, one soluble, the other insoluble in the acid, being substances which in consequence of the constancy of their properties and their composition may be considered as proximate constituents of albumin.

It might be assumed that the boiling of the albumin with dilute sulphuric acid during a limited time effected nothing but the transformation of a part of the originally coagulated substance into soluble compounds, and that the remaining insoluble matter was only unchanged or nearly unchanged albumin. But this explanation is found to be inadmissible on closer examination. For if this were the case, the residue, treated again with hot sulphuric acid during the same period as in the first operation, would have to decrease in quantity, and that in a direct ratio to the duration of the heating. This is however not the case, for the insoluble residue, the hemiprotein, is under these circumstances only very slightly and slowly changed; its weight diminishes at a ratio which, in comparison with the first diminution of the weight of the coagulated albumin, is hardly perceptible; further, the products which are obtained by the subsequent influence of acid upon hemiprotein are entirely different from those which are obtained in solution by the first cleavage.

Hemiprotein is amorphous. In certain respects it is like the amido acids (leucin etc.) combining with acids and bases without altering its constitution; it is easily soluble in alkalies,

and the solution is again precipitated by hydrochloric acid, but the precipitate is soluble in excess of acid.

By solution in dilute soda ley, accurate neutralisation with hydrochlor, and washing of the precipitate with distilled water and alcohol, hemiprotein is obtained free from incombustible ingredients. Dried at 100° it gives on quantation the following mean percentage of elements:

C 53.45; H 7.19; N 14.45.

Treatment with hot dilute sulphuric acid seems to raise the carbon in the hemiprotein. It contains sulphur, the amount of which has not been estimated; it gives the colour reactions of the albuminous substances, becomes yellow with nitric acid, red with mercuric nitrate; it dissolves in moderately concentrated sulphuric acid, and is precipitated from it by water unchanged. If to such a solution a few drops of a sugar solution are added, and the mixture is heated to 60°, a beautiful violet colouration is produced, which reminds of Pettenkofer's reaction for biliary acids.

*Chemolysis of hemiprotein by dilute sulphuric acid and boiling.*—When hemiprotein is boiled for a longer period with dilute sulphuric acid, it is split up gradually and slowly into new compounds, all soluble; amongst these are always tyrosin and leucin, and their homologues, but the most peculiar product is an amorphous colourless body of sweetish taste, soluble in water and alcohol, and precipitable by mercuric nitrate. The solubility in alcohol and the bearing with barytic hydrate, to be described lower down, distinguish hemiproteidine, as the new compound is called, from the hemialbumin which is obtained in the first chemolysis of albumin; hemialbumin is soluble in water but insoluble in alcohol. Hemiproteidine gave the following results on quantation of its elements.

			Dried at 100°	Dried at 120°
C	.	.	45.9	47.73
H	.	.	6.65	6.48
N	.	.	14.00	14.50

From these figures the formula  $C_{24}H_{42}N_6O_{12} + H_2O$  may be calculated as an abbreviated expression of the results of analysis. At 100° hemiproteidine is therefore a hydrate.

*Hemialbumin and oxyhemialbumin.*—The sulphuric acid solution which has been separated from the hemiprotein, and which contains about half the amount of albumin originally acted upon in solution, is neutralised by baryta, separated from the sulphate, and from the excess of baryta by carbonic acid, and concentrated. On the addition of an excess of alcohol to this concentrated solution a light yellow plaster-like mass, consisting of a deliquescent baryum-salt, insoluble in alcohol, is separated, while only small quantities of matter remain in solution.

*Treatment of the baryum-salt with mercuric nitrate.*—The solution in water of the baryum-salt is precipitated with acid mercuric nitrate, while the reaction of the solution is kept neutral by suitable addition of baryta water. The white voluminous precipitate is washed with hot water, suspended in water, and decomposed by hydration, the filtered solution is evaporated *in vacuo*, and yields dry, friable, amorphous, feebly hygroscopic matter, which is easily soluble in water, but insoluble in alcohol. It has the properties of the protein tritoxide of Mulder, obtained by boiling coagulated albumin with water in contact with air, or at 150° under pressure.

This body, which constitutes the greater part of the substances dissolved in sulphuric acid, does no longer yield the colour reactions which characterise the albuminous substances. Sulphuric acid does not colour it yellow, mercuric nitrate does not make it red. With sugar and sulphuric acid it gives no reaction. Its principal positive properties are that it is precipitated from its watery solution by acid mercuric nitrate, tannin, and ammoniacal lead acetate; an excess of the latter reagent redissolves the precipitate.

The mercury precipitate contains a mixture of two substances of which one contains more oxygen than the other. They can be separated to some extent by precipitation with basic and neutral ammoniacal lead acetate. The more oxygenated product has a clearly acid reaction, and seems to be more easily precipitated by basic acetate of lead.

From a number of elementary quantations it is probable that the hemialbumin, obtained by cleavage with dilute sulphuric acid, consists mainly of an amorphous body of feebly



acid properties, containing about 50 per cent. C, 7 per cent. H, and 15.4 per cent. N. From these numbers the formula  $C_{24}H_{40}N_6O_{10}$  may approximately be calculated, which differs from the soluble derivate obtained from hemiprotein by a minus of 2  $H_2O$  and a plus of  $H_2$ ;  $C_{24}H_{40}N_6O_{10} + H_2 - 2 H_2O = C_{24}H_{42}N_6O_{12}$ . Mixed with the hemialbumin is found, but only in small quantity, an acid precipitable by basic lead acetate and mercuric nitrate, whose composition can be expressed by the formula  $C_{24}H_{40}N_6O_{15}$ . This formula differs from that of the main product only by a plus of three oxygen.

The *mother liquors of the mercuric nitrate precipitate* (from the hemialbumin solution) contain a nitrogenised body, *which strongly reduces potassio-cupric tartrate, is precipitated by ammoniacal plumbic acetate, gives no precipitate with mercuric nitrate, and is probably an amidated glykose*. The sulphuric acid solution moreover contains small quantities of compounds, which are in relation to *hypoxanthin*, and can be transformed into this body, by precipitating the boiling solution with cupric acetate, dissolving the precipitate in nitric acid, and precipitating the solution with ammoniacal silver nitrate.

Hemialbumin can be further split up into relatively simple crystalloids by chemolysis with barytic hydrate.

## X.

*CHEMOLYSIS OF ALBUMIN BY FUSING CAUSTIC ALKALI. PRODUCTION OF INDOL, SKATOL, PYRROL, PHENOL AND BUTYRIC ACID. (Summary.)*

THE chemolytic action of dilute caustic alkaline leys upon albuminous substances as applied by Mulder, showed that the products of these agents were essentially the same as those of the chemolytic actions of acids, and those of the physiolytic action of putrefaction. The chemolytic power of fusing caustic potash was first applied to albumin by Liebig, and led to the discovery of tyrosin. It was subsequently used by Bopp (*Ann. Chem.* 69, 31) who observed that besides the fixed products already known a volatile body was formed, which had the odour of fæces, and was probably identical with a body of similar general properties obtained during the putrefaction of casein. Similar experiments were lately made by Kühne (*Ber. D. Chem. Ges.* 1875, 206). But instead of fusing, as Bopp had done, equal parts of caustic potash and albumin, he heated one part of albumin with ten parts of caustic potash gradually in an iron retort to a dark red heat. He obtained a body which was identical with indol in all its properties except one, namely the fusing point. These experiments were next repeated by Engler and Janecke (*Ber. D. Chem. Ges.* 1876, 1411); they heated the mixture to a still higher degree, namely to the point at which brown drops of oil were deposited in the upper part of the tube leading from the retort. They then allowed the retort to cool, added some water to its contents and distilled again. This process was repeated as long as crystalline deposits were observed in the condensers. These deposits ceased to appear in each experiment after five days of heating. They obtained in

this way from blood albumin about 0·25 per cent. of crystalline matter which had the peculiar smell of indol; its solution imparted a red colour to a chip of deal wood moistened with hydrochloric acid, and on addition of fuming nitric acid gave a precipitate of nitrate of nitrosoindol. This product, however, differed from indol in its reactions with heat; while indol from indigo fuses at  $52^{\circ}$ , the crystals obtained by these authors began partially to fuse at  $50^{\circ}$ ; the principal bulk however fused only above  $70^{\circ}$ . When they were recrystallised repeatedly from hot water the fusing point rose to from  $85$  to  $86^{\circ}$  and then became constant at that temperature, or according to Kühne at  $89$  to  $91^{\circ}$ . The recrystallisation did not, however, change either the external appearance or the smell of the crystals. The volume of the vapour of this body was to that of indol under otherwise equal conditions in the proportion of  $0\cdot9 : 1$ . As the crystals on oxydation with ozone did not give indigo, Engler and Janecke considered their product as a matter isomeric with indol, and termed it pseudindol. Nencki (*J. pract. Chem.* 17, 1878, 97) has now furnished the results of new experiments with this process, from which it appears that this pseudindol is a mixture of indol and of a similar but essentially different body, originally discovered by Secretan (*Archives des sciences de la bibliothèque universelle*. Genève, 1876; Février) in a mixture of water and albumin which had been allowed to putrefy during a period of six months, and then also met with in fæces by Brieger (*J. pract. Chem.* 17, 1878, 97) and by him termed skatol (from τὸ σκατὸς, fæces). Nencki heated the mixture of 10 potash and 1 albumin during from five to six hours, added water and heated again, and repeated this until the distillates ceased to give any red reaction with fuming nitric acid. He extracted the distillates with ether, or, after oversaturating them with hydrochloric acid, precipitated the indol and skatol by picric acid. The red needles of picrate of indol and skatol were distilled with dilute ammonia, whereby both indol and skatol were obtained in the receiver in the crystalline state. They were separated by recrystallisation from hot water; the skatol crystallised first and almost completely, while indol remained in the solution. Skatol fused at  $93^{\circ}\cdot5$ , and its solution in water gave no red precipitate with fuming nitric acid, but only

a whitish turbidity. Nencki did not obtain a sufficient quantity of skatol by this process to subject it to elementary analysis.

*Formation of oily products and of pyrrol.*—When the potash and albumin are heated quickly to a high temperature, oily products and pyrrol are formed besides indol and skatol. Small quantities of pyrrol in watery solution are easily recognised, if some drops of fuming nitric acid are added to it, or nitrous acid is produced in the solution from nitrite and acid. The mixture becomes at once dark brown, and an amorphous black precipitate is produced, which is soluble in dilute alkalies with a brown colour. Picric acid also gives with pyrrol a crystalline compound, more easily soluble in water than the picrate of indol and skatol.

*Heating the fusing mixture in an oil or paraffin bath.*—Nencki found it practicable to effect the fusion of 50 grms. albumin, and 500 grms. potash, in a glass flask immersed in an oil bath. Chemical action began at 230°; the mixture became brown, frothed greatly, and evolved great quantities of ammonia. After about an hour the high frothing ceased and the gas escaped in the form of small bubbles. The heating was continued during about five hours at temperatures varying between 260° and 290°, until the watery milky distillate ceased to come over. The flask was allowed to cool; 15 c.c. of water were added to the fused mass, and the mixture heated anew. This operation was repeated on five days until the distillates ceased to react with hydrochloric and picric acid. The picric acid precipitate produced in the united distillates acidified with hydrochloric acid weighed after drying over oil of vitriol 1.2 gm. By distillation with ammonia it yielded only 0.048 gm. skatol, which when recrystallised fused at 93° to 94° in the narrow tube.

*Treatment of the potash &c. residue. Extraction of phenol.*—The residue in the flask was dissolved in water, acidified with dilute sulphuric acid consisting of 500 grms. oil of vitriol mixed with 2 litres of water, and subjected to distillation. The strongly acid distillate had a repugnant faecal odour, a milky appearance, and deposited sulphur on standing. The filtrate from this (2400 c.c.) was neutralised with soda ley and extracted with ether. The ether was removed by distillation, and the



residue of the ether extract placed into a small retort, and distilled with dilute sulphuric acid, until the distillate on addition of bromine water did not any longer become milky. The distillate smelt of phenol, assumed a blue violet colour when mixed with ferric chloride, and with bromine water gave immediately a strong precipitate of tribromophenol, amounting to 0.152 grms. equal to 0.043 grms. as the result of the whole operation. The tribromophenol had to be purified by solution in dilute potash ley and precipitation with hydrochloric acid; the adhering impurities were perhaps a little kresol and analogous compounds.

*Extraction of fatty acids.*—The distillate, from which ether had extracted the phenol contained the volatile acids united with soda. It was evaporated to a syrupy consistency, and as it did not crystallise, was decomposed by sulphuric acid. The oily fatty acids were dried over calcic chloride to which a trace of caustic baryta had been added, and subjected to fractional distillation. The thermometer rose quickly to 150°, remained some time at 160°, and rose to 170°, when the last portion of acid distilled over. The distillate therefore consisted almost entirely of normal butyric acid: this was controlled by the quantation of a silver salt. If all acid is assumed to have been butyric, the 50 grms. of air-dry albumin, containing after deduction of ash and moisture 40.2 grms. pure albumin, yielded 14.36 grms. butyric acid, or 35.7 per cent.

*Extraction of leucin.*—The residue, from which the fatty acids had been distilled was concentrated, and the crystallising sulphate removed. The acid mother liquor was neutralised with baryta and evaporated to a syrup; it yielded 1.4 grms. of crude leucin, but no tyrosin.

*A peptone-like residue* in small quantity still gave the protein-reaction with Millon's reagent. It is long since known that as caustic potash in the dilute state produces at first so-called peptones by hydration, so the fusing potash produces peptones, but quickly splits them up into simpler products, amongst them leucin and tyrosin, then, under evolution of hydrogen volatile acid, particularly valerianic. At the same time, as we now know, indol and skatol appear. When the fusion is continued long, the amount of the peptone-like matters

is diminished, and leucin and valerianic acid are gradually changed to butyric acid. The tyrosin is completely decomposed, and in its stead phenol appears. Nencki believes that the phenol which is obtained by the fusing operation as well as that which is obtained by putrefaction from albumin, originates from the tyrosin. Whenever he obtained by either experiment phenol, he missed the tyrosin in the residues, and *vice versâ*.

Fusion with potash, like putrefaction, acts only in the first stage upon albumin by hydration. When hydrogen is developed, products of oxydation as well as reduction appear. Nencki therefore hopes that the study of the mode in which potash decomposes albumin will assist in finding out the manner in which it is decomposed by putrefaction organisms. .

## XI.

PROCESS AND PRODUCTS OF THE PUTREFACTION OF  
ELASTIN, MUCIN, GLUTIN, ALBUMIN, HEMOCHROME  
AND BLOOD. (Summary.)

GLUTIN is decomposed by physiolysis in a manner similar to that in which it is chemolysed by boiling dilute acids or alkalies; it yields no tyrosin, no indol or phenol, but of crystalloids principally leucin and glycin, and of volatile acids principally acetic. The higher albuminous substances yield indol, phenol, tyrosin and leucin, but little or no glycin, and of volatile acids principally butyric, besides some valerianic acid. There are therefore in glutin not only a lesser number of crystalloid insertions, but they are also of a less complex constitution than those contained in the higher albuminous substances. Nevertheless they belong mostly to the same homologous series as those contained in albumin. There is another difference which must not be lost sight of, namely the number of times which each radicle may occur in each kind of compound. Thus if in albumin the radicle of tyrosin be taken as one, that of leucin must be present at least six or seven times. On these quantitative proportions we have as yet few data, but they will quickly be collected when once our knowledge of the qualities of the products is completed and our methods for their quantitative estimation are perfected.

Meanwhile the methods which have yielded so much information on the substances mentioned, have been extended to a number of organoplastic substances which have not as yet been frequently studied, amongst them *elastin* and *mucin*.

*Putrefaction of elastin* (Wälchli, G., *J. pr. Chem.* 17, 1878, 71).—Elastic tissue, principally the ligamentum nuchæ of the ox, was purified in the manner practised by W. Müller (*Zeitschr.*

*rat. Med.* 10, 1861, 180). It deserves to be noticed that this method is very much like that by which cellulose is purified. The nuchal ligament is carefully dissected out of all visible connective tissue, is then torn into fibres and minced, and washed with cold water; it is then boiled with alcohol and ether to remove fats; and next boiled with water during at least twenty-four hours to dissolve all invisible connective tissue. It is further boiled with acetic acid, then with water, then with dilute caustic potash until it begins to swell, and is then finally purified by boiling with dilute acetic acid, and lastly with water. The tissue thus treated shows the specific fibres under the microscope unchanged. It is free from sulphur, and contains on an average

	W. Müller, mean of 4 analyses	Hilger, serpents' eggs
C	. 55.48 per cent.	54.68 per cent.
H	. 7.41     ,,	7.24     ,,
N	. 16.19     ,,	16.37     ,,

As regards the products obtained from elastin by *chemolysis with dilute sulphuric acid*, the data given by several authors are not very concordant. Zollikofer (*Ann. Chem.* 82, 176) found leucin to be the only crystalloid product, while Erlenmeyer and Schöffer (*J. pr. Chem.* 80, 1866, 367) found besides from 36 to 45 per cent. of leucin, also 0.25 per cent. of tyrosin. W. Müller also had obtained small quantities of tyrosin besides much leucin.

Wälcchli submitted 100 grms. elastin, purified as above described, to *putrefaction* with four litres distilled water and 5 grms. of fresh minced pancreas from the ox in a water-bath at a heat from 35° to 40°. The elastin gradually swelled and dissolved, and the reaction of the solution, neutral at first, became alkaline on the sixth day. After fifteen days the elastin all but 7 grms. dry was dissolved. The filtered liquid was treated as follows. *Distillation.* The putrid-smelling liquid was distilled from a tubulated retort, without any addition, to half its bulk. *The distillate*, which was slightly ammoniacal, was extracted with ether; the ether was distilled off, and the residue finally evaporated on a small dish: it weighed 0.0515 grms. and consisted only of a little fat derived from the ox-



pancreas, without a trace of either indol or phenol. *The residue in the retort* was mixed with 100 grms. of caustic baryta and again distilled. Nearly a litre of distillate contained 1.74 grms. of  $\text{NH}_3$ , or 1.433 grms. nitrogen. *The residue in the retort from which this ammonia had been expelled*, was freed from baryta by sulphuric acid, and again subjected to distillation. It yielded no acetic acid, but principally valerianic with little butyric acid. If the whole of the acid obtained were calculated as valerianic, it would be equal to 8.15 grms. *The residue free from ammonia and volatile acids* was evaporated on the water-bath to a thick syrup, and then mixed with absolute alcohol until a lasting turbidity was produced; it deposited crystals, which after twenty-four hours' standing were filtered from the mother liquor, and found to be a mixture of glycine and leucine in nearly equal parts. The entire weight of the crude crystals was 9.4 grms. The substances were separated by fractional crystallisation, and the glycine was identified by the analysis of its copper-compound  $2(\text{NH}_2-\text{CH}_2-\text{CO}_2)\text{Cu} + \text{H}_2\text{O}$ .

The mother liquor filtered from the glycine and leucine gave no further crystals, and after evaporation on the water-bath remained as a viscid, glue-like mass, which on treatment with caustic soda and solution of copper gave the red reaction characterising biuret.

The 100 grms. elastin, of which 93 grms. had been dissolved during the fourteen days of putrefaction, yielded therefore

Matter left undissolved . . .	7.00 grms.
Ammonia . . . . .	1.74 „
Valerianic acid . . . . .	8.15 „
Glycine and leucine . . . . .	9.40 „
Carbonic acid, saturating some ammonia, syrupy peptone- like matter . . . . .	73.71 „
Total . . . . .	100.00 „

It must be borne in mind that it is not certain whether the 7 grms. left undissolved were elastin in its unchanged state only, or contained other less putrefiable tissue-elements such as characterise the nuclei of cells and their residues. Seeing that

nearly three quarters of the elastin employed were transformed only into the syrupy peptone-like matter, which was not studied any further, the experiment will have to be continued. But the matters actually obtained, and the absence of aromatic radicles, characterise elastin as related to the gelatinogenous tissues; but it is again different from these and resembles the higher albumins by yielding in the operation for volatile acids the higher valerianic, while gelatin yields the lower acetic acid.

*Putrefaction of mucin.*—Wälchli (*loc. cit.* p. 75) prepared the mucin used in his experiment from the large vineyard snails, *Helix pomatia*; the crushed and minced snails were ground up with glass powder, and the paste on a felt funnel was extracted with boiling water. The yellowish filtrate was mixed with an excess of strong acetic acid, and the precipitated mucin was washed with water by decantation, then collected on a filter, when in a solid state extracted with ether to remove fat, and dried. The mucin thus prepared was a blackish tough mass. 223 grms. of the air-dry substance, corresponding to 163 grms. mucin dried at 120°, were digested with 4 litres of water and 5 grms. of ox-pancreas in a water-bath at 35° to 40°. On the 9th day the mucin, all but 6·8 grms. dry, had dissolved. The fluid, of strongly putrid odour, was subjected to distillation. The distillate gave no reaction for indol with fuming nitric acid. It was saturated with soda ley, and extracted with ether. This solvent left a small amount of an oil, which did not crystallise on long standing, and had the smell of the volatile principle obtained from human excrements and from those of dogs and from offensive-smelling products of disease (skatol,  $C_{10}H_{11}N$ ). The oil was boiled with water, which dissolved a small portion of it. The hot filtrate became milky on cooling, and after some hours deposited crystals of indol. The filtrate from these crystals was diluted with some water, mixed with a few drops of potash ley, and subjected to distillation from a small retort, until the distillate gave the reaction for indol. The residue in the retort was now oversaturated with dilute sulphuric acid and again distilled. The distillate smelled of phenol, and with bromine water gave white, crystalline tribromophenol. The volatile product therefore contained both indol and phenol, and

a peculiar-smelling principle in prevailing amount, which was not suitable for further analysis.

The liquid, from which these matters had been expelled, was now mixed with baryta, and again distilled it yielded 3.4 grms. of  $\text{NH}_3$ , equal to 2.87 grms. N. The acidified liquid gave almost pure butyric acid, amounting to 12.3 grms.

The residue, now free from ammonia and acids, contained an uncrystallisable, sweet-tasting substance, which reduced potassio-cupric tartrate, decomposed cupric and barytic carbonate, and formed with these bases amorphous salts precipitable by alcohol. The author believes this body to be the same as the sugar-like body which was first obtained from mucin by Eichwald. He lost his preparation, stored in a dish of 'toughened glass,' by the said glass spontaneously bursting up into little splinters and spilling the contents.

*Changes in the appearance of blood-corpuscles during the decomposition of blood in the presence of Bacillus subtilis and of different gases.*—The life of bacteria in gases has been investigated by Grossmann and Mayerhausen (*Archiv Physiol.* 15, 245). Oxygen caused the bacteria to move strongly and change quickly in form, while ozone had the opposite effect, and when applied in a concentrated state destroyed the bacteria almost instantaneously. As several oxydations by ozone have the same character as oxydations of the same substances in the animal economy (e.g. that of uric acid, which yields in both cases allantoin and urea) and as it had been stated by A. Schmidt, that the blood-corpuscles ozonise oxygen in contact with them, Kaufmann (*J. pr. Chem.* 17, 1878, 70) examined the bearing of putrefaction-ferments in oxygen in the presence of red blood-corpuscles. The latter were enclosed in a so-called moist capillary chamber, a double capillary lens, invented by Recklingshausen and manufactured by Geissler. The experiment was in each case made as follows: a few drops of defibrinated blood were sucked into the chamber, then mixed with about an equal bulk of liquid containing bacteria in suspension, and after complete mixture in the chamber most of the liquid was again removed from it, so that only the capillary space and its immediate surroundings retained any liquid. Through the chamber thus supplied, oxygen, passed through potash and

sulphuric acid, was conducted. The blood operated upon was in one experiment from the frog, in four other experiments from rabbits. The uniform result was, that while bacilli in putrid liquid without blood, maintained in oxygen, remained moving and unchanged, bacilli of the same breed mixed with blood became after a longer or shorter time perfectly motionless. Therefore, although the oxygen is perhaps not transformed into ozone by the blood-corpuscles, its action in the moist chamber is such as to produce an effect similar to that of ozone upon bacilli.

The corpuscles of the blood of the frog remained unaltered in shape in the moist chamber, though in contact with putrid ferment bacilli during five days, oxygen being constantly passed through the chamber; on the sixth day the nucleus was granulated; on the eighth day many corpuscles were completely decolourised and appeared only by their nuclei and stromata. The changes were not followed further.

The changes in the blood-corpuscles of rabbits did not appear to differ much, but to be slower than those which are observed when the blood is placed under the microscope on an ordinary slide and covered with thin glass.

The author thinks that oxygen in presence of blood-corpuscles, and somehow modified by them, kills, or keeps inert and prevents the development of, the micro-organisms, the germs of which he believes, after Béchamp (*Des Microzymas* &c. Paris, 1875) and Tiegel (*Archiv Pathol.* 60, 453), to be present in nearly all tissues of the healthy body. When the passage of oxygen through the moist chamber was stopped, the bacilli regained their motility and power of multiplication. These bacilli therefore obey conditions of life the opposite of those by which the *Bacillus anthracis* abides, which thrives well in oxygenated blood, but dies in putrefying blood or other liquids.

*Putrefaction of blood-corpuscles and of blood in the presence of bacilli.*—Some experiments on these processes were made by Kaufmann (*J. pract. Chem.* 17, 1878, 90). In the two first experiments two litres of blood were defibrinated and mixed with their tenfold volume of a mixture of one volume of saturated solution of common salt and nine volumes of water. After 40 hours the corpuscles had settled and the supernatant fluid was



decanted. The muddy deposit was diluted with five litres of water, mixed with 5 grms. of minced ox-pancreas, and digested on the water-bath at 40°. After 24 hours the mixture exhaled a putrid odour, and a thin reddish-brown crust floated on its surface. The blood-corpuscles were all destroyed (by the water?) and the solid matters visible consisted of micro-organisms, particularly heaps of zoogloea. The crusts were examined on the second day and found to consist of hematin. On the fifth day the liquid contained mainly bacilli; on the tenth day cocci were added. From this time to the sixteenth day the micro-organisms consisted of cocci and bacilli in equal parts. The surface of the fluid exhibited much hematin in crusts, but the liquid still contained hemochrome in solution.

The liquid subjected to distillation yielded a little indol, but no phenol. The residue in the retort gave leucin and tyrosin. The mother liquor contained fatty acids, combined with ammonia and peptones. These were unfortunately not examined any further. In a second experiment with the corpuscles from two litres of blood, the pancreas was omitted, and the results did not differ from those of the first experiment, although they were perhaps attained at a greater expenditure of time (29 days).

The third experiment was made upon three litres of blood with five grms. of ox-pancreas. The micro-organisms multiplied rapidly; after five days the blood-corpuscles had all disappeared. After six weeks of putrefaction the liquid was distilled. It yielded much indol and phenol, but no tyrosin, little leucin, fatty volatile acids, ammonia, and peptones. As, before being boiled, the liquid still contained some hemochrome, the author comes to the conclusion that hemochrome possesses great stability and power of resistance against the attacks of micro-organisms. As regards the use of pancreas the author comes to the result that the experiments without it were just as successful as those with it, because, as he thinks, the air of his laboratory was provided with abundance of bacteria or their germs.

## XII.

ON THE ALKALOIDS OF THE HUMAN URINE. (*Consolidated Account of Researches. From the Pathological Institute.*)

ALKALOIDS may be defined as bodies derived from typical ammonia, by substitution of compound radicles for one or more of the atoms of hydrogen; the radicles may be homogeneous or heterogeneous, the typical ammonia may be one, or may be present several times over, constituting a multiple type, which gives rise to the possibility of countless substitutions by compound radicles. The best known representatives of this class are the so-called amines and ammonium bases, obtained synthetically; but the most important are the alkaloids of vegetable origin and great poisoning or healing power, such as the principles extracted from opium, cinchona bark, and nuxvomica. Of these bodies above a hundred are at present known to science, and when we add the bases derived from the animal economy and obtained by other means, we arrive at a number exceeding two hundred. It appears, however, that the definition has hitherto been too narrow, just as the modes of searching for alkaloids were far too one-sided and limited. For alkaloids were supposed to be very insoluble, in water at least, and it is not long since this error was recognised when a few highly soluble alkaloids such as colchicine were discovered. It will now be necessary further to widen the definition of alkaloids, so that the bodies, of which the alkaloid from brain-matter is a representative, may be ranged under it. I have shown with regard to the phosphorised ingredients of the brain that they possess both basic and acid properties at the same time. The same is the fact with regard to the new alkaloids to be described; they possess most of the combining-powers

which are considered to be characteristic of alkaloids, but at the same time they possess combining-powers characteristic of acids. Such alkaloids I have extracted from the muscular and brain tissue in great quantity, from several other tissues of organs, particularly the liver, and from secretions, such as milk and urine.

*Proceedings for the isolation of the alkaloids of the urine.*

*First modification; new alkaloid: reducin.*—Fresh healthy human urine was shaken with a little animal charcoal to collect the mucus and epithelial elements, filtered, strongly acidified with sulphuric acid, and then precipitated by phosphomolybdic acid. The collected precipitate was washed with water containing a little sulphuric acid. It was then decomposed with hot baryta-water in slight excess; this excess was removed by carbonic acid and boiling, and the filtrate evaporated to a small bulk. On cooling it formed a deposit, which was proved to be *pure urate of baryum*. It was specially proved that it contained no hypoxanthin. The yellow filtrate contained much urochrome, giving on decomposition with hydrochloric acid and boiling uromelanin, uropittin, and evolving smell of omicholic products. It was therefore treated with neutral lead acetate, after this with the basic lead acetate, and ultimately with this and some ammonia. The united precipitates were further treated, but the results were not so satisfactory as those of the copper process to be stated below. The filtrate from the lead precipitates was freed from excess of lead by hydrothion, and evaporated to dryness while being stirred on the water-bath. The residue was treated with boiling absolute alcohol and the decoction filtered hot. A voluminous baryum salt remained on the filter, while a yellowish matter dissolved in the alcohol. The former was *reducin-baryum*, the latter mainly *kreatinin*.

*Reducin-baryum.*—It was easily soluble in water, and after burning left baryum carbonate. On addition to the solution of some nitric acid and silver nitrate a precipitate was produced which immediately became dark and black in the cold. With mercurous nitrate and nitrite it gave an immediate black precipitate; with mercuric chloride it gave a white precipitate which was not changed by boiling; with cupric acetate and

boiling, it gave a flaky precipitate which became brown; with Fehling's solution and boiling it gave no reduction.

Analysis of the baryum compound dried at 100°.

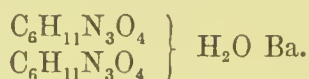
0.4540 grms. gave 0.4520  $\text{CO}_2$  = 27.092 per cent. C, and 0.1750 grms.  $\text{H}_2\text{O}$  = 4.282 per cent. H.

0.2628 grms. gave 33.3 c.c. gas normal = 15.850 per cent. N.

0.4490 grms. gave 0.1940  $\text{BaSO}_4$  = 25.400 per cent. Ba.

		Per cent.	÷ At W.	÷ Ba. = 1.
C	. .	27.092	2.257	12.2
H	. .	4.282	4.282	23.1
N	. .	15.850	1.132	6.0
Ba	. .	25.400	0.185	1.0
O	. .	27.376	1.711	9.2

If the substance be considered as a monobasic acid, these figures lead to the formula



If it be considered as a dibasic acid they lead to  $\text{C}_{12}\text{H}_{24}\text{N}_6\text{O}_9$  as the formula of the free substance. Of this the molecular weight would be 396, while the molecular weight pointed to by the quantity of baryum is 402.

The foregoing figures and formulæ are given with the reserve imposed by the limited quantity of material; but small changes in proportions can detract nothing from the essence of the new facts. The isolation of *reducin* has been effected by the application of a reagent of special action on alkaloids. This is the principal proof of the alkaloidal nature of the new body; that it combines with bases after the manner of acids need cause no surprise, as there are many substances known to chemistry which have similar properties; the best known amongst these are the amido-acids, like glycine.

*Kreatin and kreatinin.*—The alcoholic solution filtered from the *reducin* just described, was evaporated and the residue extracted with little absolute alcohol to remove kreatinin and leave kreatin; the residue was dissolved in hot water, and decolourised by boiling with animal charcoal. It was then allowed to crystallise, and yielded three successive crops of crystals of



kreatin. The air-dry crystals had the appearance of kreatin, and their nature was verified by a determination of the water of crystallisation.

0.4596 grms. dried *in vacuo* over sulphuric acid, lost 0.5076 grms. or 12.53 per cent. H O. The theory of  $C_4H_9N_3O_2$ ,  $H_2O$  requires 12.08 per cent. The crystals were therefore kreatin. It is known that kreatinin, when freed from its combinations and particularly in warm baryta-water, is easily transformed into kreatin, and these bodies are therefore nearly always obtained side by side in certain proportions.

The alcoholic extract contained kreatinin and the peculiar alkaloid which gives an insoluble compound with zinc to be described lower down, and a last fourth new alkaloid which remains in the ultimate mother liquor and is precipitated from alcoholic solution by platinic chloride. The compound is decomposed during resolution with water, but a stable platinum compound results, which contains an aromatic ingredient. It has not yet been analysed.

*Second modification of proceedings for the isolation of alkaloids of the urine. Second and third new alkaloid.*—In the researches now to be described the processes adopted were slightly modified. The raw material was filtered only, and not treated with even the smallest quantity of animal charcoal, as it was found that the agent removed considerable quantities of the matters to be studied. It was next strongly acidified with sulphuric acid in the following manner and proportions. Fifty cubic centimetres of oil of vitriol were poured into 100 c.c. of water, and the resulting 150 c.c. of dilute acid were poured into a litre of filtered urine. This mixture was now fully precipitated with a solution of phosphomolybdate of soda acidified with a sufficient excess of sulphuric acid to represent a deep yellow solution of phosphomolybdic acid, containing 200 grms. of the solid acid in each litre. In each operation the quantities of phosphomolybdic acid solution required were found by preliminary experiment, and the completion of the actual operation on the large scale was ascertained by the mother liquor of the precipitate giving no further reaction with either sulphuric or phosphomolybdic acid.

The collected buff-coloured precipitate was washed with

water containing about five per cent. of sulphuric acid. This was effected by washing the precipitate into a large stoppered bottle by means of the spray from a continuous pressure wash-bottle (described in another place), then adding a large amount of acidified water, shaking the mixture strongly, allowing the precipitate to settle, and removing the washing water with the syphon. By repetition of this treatment the precipitate could be almost entirely freed from alkalis and chlorine.

The precipitate placed in a flask with some water and warmed in a water-bath was now decomposed with hot concentrated solution of caustic baryta in slight excess; the point at which the excess of the alkali is present is indicated by a change in the colour of the liquid from blue to yellow, and in the colour of the precipitate from greenish buff to pale bluish grey. The excess of caustic baryta was now neutralised by carbonic acid conducted into the flask with the fluid and phosphomolybdate of baryum kept continuously hot in the water-bath. When a filtered sample was no longer affected by carbonic acid, the solution was separated from the precipitate by filtration with the usual precautions.

*Volatile bases.*—In some of the experiments the *volatile bases* which are always set free on the first addition of the baryta were fixed by being passed through a vessel containing hydrochloric acid. The solution was evaporated, the chlorides again distilled with caustic soda, and the peculiar and fetid smelling distillate neutralised with dilute sulphuric acid. The dry sulphates gave a portion to absolute alcohol, which contained a compound ammonia, probably trimethylamine, while another portion remained insoluble in absolute alcohol, and was recognised as ammoniac sulphate.

*Further proceedings for separation of alkaloids.*—The liquid from which all volatile alkalis had been expelled by heat and the carbonic acid treatment was concentrated to a convenient bulk, allowed to cool, and after some standing filtered from the small deposit of *urate of baryum*, which was mostly formed. The uric acid is probably present in the phosphomolybdic precipitate as an admixture felled by the sulphuric acid, and not in combination. It is small in quantity and the trace which may possibly remain in solution after deposition of

the baryum salt does not appear in any subsequent operations, and at one stage of the subsequent proceedings is necessarily destroyed.

There remains now a solution of mixed alkaloids, coloured yellow, and containing baryta in solution not removable by carbonic acid. I have elsewhere described the application to this solution of lead acetate both basic and neutral, and the further treatment of the lead precipitates, resulting in the isolation of a body having the reaction of xanthin, but differing from it in the composition of its silver salt. It was shown that there were at least two bodies present, imitating the bearing of xanthin with silver nitrate and ammonia, but that the second one differed from xanthin not only in the amount of silver contained in the argentide, but also by the solubility of the argentide in excess of concentrated caustic ammonia.

In the experiments here to be related, the united alkaloids were not treated with lead salts, but with cupric acetate, which produced a voluminous greenish, later buff-coloured precipitate. (On the application of acetate of copper to urine for the precipitation of a base described by Strecker as sarcin, sarkin, hypoxanthin, but also suspected to be guanin, see my *Pathology of the Urine*, first edition, 1858, Appendix.) The change of the precipitate to buff colour was found to be due to a reduction of the cupric to cuprous salt; this occurred in an acid liquid, and did not occur when the liquid was made alkaline; the reduction became very copious when the mixture of liquid and copper precipitate was heated; in that case the precipitate became purely yellow. When the precipitate was quickly filtered from the solution in which it had been formed, and washed with water, so as to be free from mother liquor, it could be treated with hot water without changing its colour; but it always contained cuprous salt from the beginning.

This cuproso-cupric precipitate was decomposed with hydrothion, and the filtered liquid boiled and evaporated. At a certain stage of concentration it deposited crusts and pellicles, and then was allowed to cool, when it deposited a pulverulent body. All deposits were separated from the deeply coloured mother liquor, and treated as will be immediately described. The deeply coloured mother liquor contained yet much of the

pulverulent body, and was separately treated for its separation by evaporation and cooling; but its main ingredient was urochrome, precipitable by ferric chloride, and giving the same reactions as that precipitated by the lead process.

*Second new crystallised alkaloid.*—The deposits were washed with cold water, and redissolved in hot, and filtered through Swedish filtering paper, as often as was necessary to free them from all matters which became gradually insoluble in the course of that treatment. The granular matter gradually assumed a crystalline appearance, and after the removal of several mother liquors became perfectly crystallised in rhombic scales of the lustre of mother of pearl. When the crystals were homogeneous under the microscope and perfectly white they were analysed.

- (1) *Combustion in vacuo.*—0.0222 grm. dried during 48 hours *in vacuo* over  $\text{H}_2\text{SO}_4$ , was burned with cupric oxyde *in vacuo* and gave: Total gas=28.5 c.c.; after KHO=7.6 c.c.; KHO column=3.7 m.m. Hg. Hg column=82 m.m.; B=764.5; T=25°. Therefore total normal gas=25.45 c.c.; total  $\text{CO}_2$ =19.45 c.c.; N=6.00;  
 Vol. of N : Vol. of C=1 : 1.62  
 or =4 : 6.48

or in percentages equal to 46.95 C and 33.81 N.

- (2) Nitrogen quantation by CuO and  $\text{CO}_2$  mixture 0.0988 grm. gave 29.5 c.c. gas. KHO column=5.2 m.m. Hg; B=759 m.m.; T=21°; therefore=26.5 c.c. gas normal=33.56 per cent. N.
- (3) 0.3704 grm. recrystallised from alcohol, and dried at 100°, burned with cupric oxyde gave 0.1556  $\text{H}_2\text{O}$ , equal to 4.69 per cent of hydrogen.

		$\text{C}_7\text{H}_8\text{N}_4\text{O}_2$		
Theory.		Found.		
		1.	2.	3.
7 C . .	84	46.67	46.95	—
8 H . .	8	4.44	—	4.69
4 N . .	56	31.11	33.81	33.5
2 O . .	32	17.78	—	—
	180	100.00		



By combustion of pure theobromin from cacao *in vacuo* a mixture of gases should be obtained in which  $\text{CO}_2 : \text{N} = 14 : 4$ ; the proportion obtained in the analysis of the new base was  $= 12.96 : 4$ . The excess of nitrogen may perhaps be due to a slight admixture of hydrogen with the nitrogen gas. For at that period I had not yet ascertained the error introduced into the nitrogen quantation, particularly in the combustion *in vacuo*, by the hydrogen occluded by the copper reduced in the hydrogen current. This error was first eliminated by a research communicated to the Chemical Society, and published in its *Journal*, September 1876, under the title: 'On the Estimation of Hydrogen occluded by Copper, with special reference to Organic Analysis,' by J. L. W. Thudichum and Henry W. Hake. The final decision of this point must be reserved for further research.

This body, therefore, has very nearly the composition of theobromin, the alkaloid from cacao. Like this it is sublimable; fusing just before yielding the vapour which condenses in white clouds; it is tasteless on the tongue at first, but evolves a bitter taste after some time, which is never very intense; it is easily soluble in absolute alcohol, more in hot than in cold; and forms salts with acids. But it is not identical with theobromin, as it is much more soluble in water than this base, and with nitrate of silver in dilute nitric acid solution does not yield the crystallised double salt which theobromin from cacao so easily produces. On further comparison with theobromin it appears moreover that this base is not precipitated to any extent by cupric acetate, whereas the new base forms with this reagent a precipitate which is completely insoluble in even boiling water.

From xanthin it is distinguished by its easy solubility in hot water; solubility in alcohol, in which xanthin is quite insoluble; and by its subliming without decomposition; further by its elementary composition; for xanthin,  $\text{C}_5\text{H}_5\text{N}_4\text{O}_2$ , has

C . .	39.48
H . .	2.63
N . .	36.84
O . .	21.05
	<hr/>
	100.00

figures which differ so much from those above quoted for the new base as to exclude absolutely any probability of identity.

*Study of kreatinin obtained by the phosphomolybdic acid process.* (a) *Reaction with ferric chloride.*—A faintly yellow solution of ferric chloride is coloured dark red by a dilute solution of pure kreatinin. The depth of colour is increased by boiling.

Chloride of zinc-kreatin produces the same reaction.

(b) *Compounds of kreatinin with gold chloride.*—A quantity of kreatin, obtained by the phosphomolybdic process, was treated with sulphuric acid for the purpose of being transformed into kreatinin, and the product was three times recrystallised from alcohol, the white plates of kreatinin sulphate were dissolved in water, and decomposed accurately with barytic chloride. The neutral kreatinin hydrochlorate was concentrated and mixed with a concentrated solution of gold chloride. No immediate precipitate ensued. On standing warts of a compound formed, which was indistinctly crystalline. The compound recrystallised from hot water seemed less soluble than the original mixture, and became turbid at once on slight cooling. On standing some metallic gold was deposited. The reformed compound was apparently very indistinctly crystalline, not crystallised. It was collected, washed, and dried *in vacuo*.

*Analyses :*

- (1) 0.0836, after combustion, left 0.0400 metallic gold = 47.84 per cent. Au.
- (2) 0.1394 gave 12.5 c.c. gas at B = 765 m.m. T = 18°; KHO column = 110 m.m.; equal to 11.39 c.c. gas normal = 10.25 per cent. N.
- (3) 0.994 by fusion with caustic soda and nitre gave 0.0484 Au, equal to 48.69 per cent., and 0.0903 AgCl, equal to 22.46 per cent. Cl.

A preparation of kreatinin, which had been obtained directly by the phosphomolybdic acid process, and been purified by recrystallisation from absolute alcohol, was dissolved in excess of hydrochloric acid, and mixed with auric chloride solution. Crystals formed, which were separated and pressed. The mother liquor was yet three times treated with gold chloride, and yielded altogether four crystallised deposits, 3.2 grms. in weight. The first and second crystals were recrystallised.

*Analysis:*

0.1043, fused in caustic soda and nitre, gave 0.0449 gold, or 43.04 per cent. Au, and 0.1332 AgCl, or 31.59 per cent. Cl.

The salt could not be dried at a higher temperature; heated to near 100° during one hour, it became dark in colour; 0.1662 lost 0.0052; 0.1602 burnt left 0.0718, or 44.81 per cent. Au.

The same salt by fusion gave 44.41 per cent. Au.

The third crystals were not recrystallised but tested separately; 0.1400 grm. dried *in vacuo*, left 0.0602 gold on combustion, equal to 43.00 per cent. Au.

A similar preparation was obtained by dissolving some kreatinin zinc chloride in hydrochloric acid, and adding auric chloride; it weighed 1.5 grm.

The whole of the products were dissolved in warm water, filtered from some insoluble matters, and the solution placed in a vacuum over sulphuric acid. A crystalline deposit formed, consisting of spherical masses of crystals. On the salt being dried *in vacuo* over sulphuric acid, it became slightly discoloured, though it was kept in the dark.

*Analyses:*

- (1) 0.1552 left 0.0726 metallic gold = 46.77 per cent. Au.
- (2) 0.3734 gave 33.0 c.c. gas at B. 757 m.m.  $T = 15^{\circ}$ ; KHO column = 113 m.m. equal to 30.16 c.c. gas normal, or 10.46 per cent. N.

Consequently by recrystallisation of the dry salts from pure water the percentage of gold increased from 43.0 to 46.77, and the nitrogen rose above the quantity theoretically to be expected.

Some further gold chloride compounds were made with kreatinin obtained from zinc chloride compound by silver oxyde: excess of hydrochloric acid and of gold chloride were added. Three crystallisations were obtained in succession:

*Gold Quantations:*

No. (1) 0.1936 left 0.0832, or 42.98 per cent. Au.

No. (2) 0.1322 left 0.0568, or 43.12 per cent. Au.

No. (3)  $\left\{ \begin{array}{l} a. 0.0810 \text{ left } 0.0348, \text{ or } 42.91 \text{ per cent. Au.} \\ b. 0.0614 \text{ left } 0.0624, \text{ or } 42.99 \text{ per cent. Au.} \end{array} \right.$

It was thus evident that kreatinin in the presence of excess of hydrochloric acid would yield a hydrochlorate aurochloride in crystals, but that this salt (except in one case, in which only a small amount of crystals was obtained from a larger amount of solution) could not be recrystallised from water without losing hydrochloric acid and forming a deposit of metallic gold. When neutral kreatinin hydrochlorate was mixed with auric terchloride, the hydrochloric acid was also ejected, and a compound free from hydrochloric acid obtained. A considerable excess of hydrochloric acid was required to insure a good crystallisation of the double salt. This bearing is shown conspicuously by the following synopsis of the several theories and results of analyses :

*Kreatinin Hydrochlorate Aurochloride from Solution with excess of Acid.*

Theory.			Found.				
Atoms.		in 100.	1.	2.	3.	4.	5.
4 C	48	—	—	—	—	—	—
8 H	8	—	—	—	—	—	—
3 N	42	9.27	—	—	—	—	—
O	16	—	—	—	—	—	—
Au	196.7	43.45	43.00	43.04	42.98	43.12	42.95
4 Cl	142	31.36	31.59	—	—	—	—
		452.7					

Preparation (1) and (2) united and recrystallised from water gave

N	.	.	10.46
Au	.	.	46.47

*Kreatinin Aurochloride from Neutral Hydrochlorate.*

Atoms.		in 100.	Found.		
			1.	2.	3.
4 C	48	11.53	—	—	—
7 H	7	—	—	—	—
3 N	42	10.09	—	10.25	—
O	16	—	—	—	—
Au	196.7	47.26	47.84	—	48.69
3 Cl	106.5	25.58	—	—	22.46
		416.2			



All salts obtained and analysed in the foregoing were now united and recrystallised with addition to the solution of hydrochloric acid. The first deposit of crystals was dried *in vacuo* and analysed.

(1) 0.1770 burned left 0.0768 = 43.39 per cent. Au.

(2) 0.3426 fused with soda and nitre, &c., gave 0.1507 = 43.98 per cent. Au and 0.4308 AgCl = 31.12 per cent. Cl.

(3) 0.1662 gave 13.0 c.c. gas, normal, = 9.25 per cent. N.

(4) 0.2656 gave 0.1058 CO<sub>2</sub> = 10.86 per cent. C, and 0.0468 H<sub>2</sub>O = 1.93 per cent. H.

### *Synopsis of Results.*

Theory.		Found.			
Atoms.	Per cents.	1.	2.	3.	4.
4 C	10.60	—	—	—	10.86
8 H	1.77	—	—	—	1.93
3 N	9.27	—	—	9.25	—
O	—	—	—	—	—
Au	43.45	43.39	43.98	—	—
4 Cl	31.36	—	31.12	—	—

It is thus evident that kreatinin forms a compound with gold chloride of the formula C<sub>4</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub>AuCl<sub>3</sub>, which is not very well defined, owing to its instability.

This compound is formed when kreatinin hydrochlorate and gold chloride are mixed together, the molecule of hydrochloric acid being ejected and remaining dissolved in the mother liquor.

The same compound is formed as often as it is attempted to recrystallise the salt C<sub>4</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub>HClAuCl<sub>3</sub> from water.

The most stable and best-defined salt is the kreatinin hydrochlorate aurochloride of the formula just quoted; this is formed only in the presence of a considerable excess of free hydrochloric acid in the solution from which it deposits; if the excess of hydrochloric acid over and above the proportion required to form a hydrochlorate is insufficient, a mixture of the aurochloride with the hydrochlorate aurochloride is produced.

*Third new alkaloid giving insoluble yellow compound with zinc (oxyde), (C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O, ZnO).*—The precipitate by zinc chloride in the absolute alcohol solution of the alkaloids (from which the new crystallised alkaloid described and

kreatinin and kreatin had been removed to a great extent by precipitation and crystallisation) was flaky and adhesive; it was allowed to stand in the mother liquor for twelve hours, until the latter was clear, and then separated. The precipitate, which had become hard, dissolved in water only partially, leaving a *yellow zinc compound* insoluble (Preparation I.). The watery mother liquor was evaporated, and yielded several crops of crystallised kreatinin zinc chloride. At last it became thick and syrupy, and contained a deposit of crystals and flakes, which could not be isolated from the syrup. The whole was therefore again poured into a large excess of water, and more water was added as long as the mixture became turbid. The yellow precipitate was filtered off (Preparation II.). The same process was repeated upon the mother liquor and yielded a third product (Preparation III.). All three preparations were dried *in vacuo*.

The precipitates were easily soluble in acids, such as acetic and sulphuric; in these solutions phosphomolybdic acid gave a copious precipitate; the substance had therefore preserved its character of alkaloid. It was easily soluble in excess of caustic ammonia. On being heated it evolved a repugnant smell, and left zinc oxyde. It was quite insoluble in water, and could be boiled with it apparently without undergoing any change.

From preliminary quantations it appeared that the body gave up a certain amount of water *in vacuo*, and then gradually further quantities up to a temperature of 170°, at which its weight became stable.

0.5600 grm. taken for experiment:

Temperature	Time	Loss
100°–110° . .	20 hours	·0519
130° (about) . .	2 „	·0045
135°–140° . .	2½ „	·0096
150° . . . .	2½ „	·0052
150°–170° . .	2½ „	·0024
170°–175° . .	4½ „	·0002

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Total loss . . . 0.0738

or 13.18 per cent. of substance.

A second experiment upon another quantity of substance gave precisely the same result.

The preparations were mixed and subjected to a careful qualitation. The elements found were carbon, hydrogen, nitrogen, oxygen, and zinc, with traces of calcium, copper, and iron. The last three elements were evidently non-essential admixtures or impurities, and were eliminated.

The quantations led to the following results :

		Per cent.	At. Weights
C	. .	33·34	72
H	. .	3·90	9
N	. .	18·91	42
ZnO	. .	36·84	81
O	. .	7·01	16
		<hr/>	<hr/>
Total		100·00	220

These data lead to the empirical formula  $C_6H_9N_3O, ZnO$ .

It will at once be seen that this is the formula of reducin, minus two atoms of oxygen, plus a molecule of zinc oxyde. Although the compound had been precipitated from the alcoholic solution of the mixed alkaloids by zinc chloride, yet it was quite free from chlorine, not a trace being discovered by a quantation. Consequently the zinc chloride compound of this alkaloid undergoes the same change as the kreatinin hydrochlorate auric chloride, in the presence of water; it loses the hydrochloric acid completely, and becomes insoluble. The form in which the zinc may be present has in the foregoing been assumed to be that of oxyde; but this is only hypothesis, and further analysis must show whether or not it may be present as metal replacing hydrogen, and therefore lead to the formula  $C_6H_9ZnN_3O_2$ .

The foregoing process was repeated upon 65 litres of material, which had been treated with zinc and hydrochloric acid. The zinc compound was obtained, but like the other alkaloids, in apparently much smaller quantities than from material which had not been reduced by zinc and hydrochloric acid.

One hundred parts of the air-dry compound, powdered, lost *in vacuo* and over sulphuric acid, and then by heating to  $170^\circ$ ,

15.73 parts in weight; the loss assumed to be water only; of this loss almost two-thirds occurred in the vacuum, a little more than one-third at between  $100^{\circ}$  and  $170^{\circ}$ ; the total loss of water almost amounts to two molecules, so that the formula for the air-dry substance might be written  $C_6H_9N_3O$ ,  $ZnO + 2H_2O$ .

The phosphomolybdic acid process is thus shown to remove from urine at least six fixed alkaloids besides the volatile bases. Of these alkaloids *kreatinin* is well known; *urochrome* is now shown for the first time to be an alkaloid, and to be precipitable by ferric chloride; *reducin* is distinguished by a baryum-compound and a remarkable reaction; the *second new alkaloid* is distinguished by its crystallisability, and by features which resemble those of the theine group; the *third new alkaloid* gives an insoluble compound with zinc; and the *fourth new alkaloid* gives a compound with platinic chloride, and contains an aromatic nucleus.

The physiological quantities of these substances are probably not so small as one might be induced to believe from the small quantities actually isolated even from considerable quantities of material by the above process; for the phosphomolybdic precipitate is not very insoluble, and consequently some is lost in the voluminous mother liquors; these had in the first instance to be operated upon without the employment of heat; but evaporation may in future be tried with certain precautions without fear of destroying the substances above described. The phosphomolybdic acid undergoes some reduction in the urine, which becomes deep green from the admixture of blue oxyde with its naturally yellow colour. It is probable that a portion of the *reducin* contributes to this effect, and after its oxydation escapes the further steps of the process. Then the baryta process is undoubtedly unwieldy, and likely to produce loss, particularly of bodies of the xanthin group. On the whole, however, the process yields a considerable amount of new and useful information.



## XIII.

*THE PROPERTIES AND METAMORPHOSES OF SOLUBLE ALBUMIN. (Summary and Additions.)*

ALBUMIN, popularly so-called, is the well-known white of egg. It is obtained by breaking a fresh hen's, or other bird's egg, separating off the yolk and its envelopes (chalazæ), keeping the chalazæ and all mucous and ropy parts away with particular care, shaking the white in a bottle with some fragments of glass, and filtering the liquid through calico, lastly through paper by means of a pressure-filter.

The substance thus prepared contains, besides albumin chemically so-called, some albuminate and plasmin (paraglobulin), traces of fats, and soaps, grape sugar, and a quantity of salts amounting to about three per cent. of the solid residue.

Crystals of an alleged albuminous body were observed during the drying of albumin by A. Boettcher (*Arch. Path. Anat.* 32, 1865, 525 and 533). They were found in different organs and liquids, and were variously interpreted by different authors, but according to Schreiner (*Liebig's Ann.* 194, 1878, 73), they are the phosphate of an organic base,  $\text{PO}_4\text{H}_2(\text{C}_2\text{H}_5\text{N})_4$ . At 100 it loses  $3\text{H}_2\text{O}$ . (See SPERMATIN.)

Oonin was separated from white of egg by freezing by Couerbe (*J. Pharm.* 15, 497, *Ann. Chim. Phys.* 41, 323). Liebig in his lectures mentioned this experiment and termed the matter a mucous substance; when it is removed the white of egg is quite liquid and filterable.

Some differences between the whites of the eggs of fowls and those of pigeons were discussed by Jahn (*N. Br. Arch.* 37, 259; Berzel. *Jahresb.* 25, 875).

Observations on the whites of wild birds' eggs were made at the Pathological Institute, and will be communicated on a future occasion.

*Modes of purifying albumin.*—Among older modes is that of Würtz (*Compt. rend.* 18, 700) who precipitated diluted and filtered white of egg with basic lead acetate not in excess; suspended the washed precipitate in water, and passed carbonic acid through the liquid; filtered, precipitated traces of lead from the filtrate by a few drops of aqueous hydrothion; heated the clear brown liquid to 60°, whereupon the sulphide of lead was precipitated with the first flocks of albumin. He then filtered again and evaporated the filtrate at 50°. The product still contains salts on its own showing, as pure albumin is not precipitable by boiling; it also contains acetic acid, and is precipitable by ammonia. Von Wittich's process (*J. pr. Chem.* 73, 18) produces an altered albumin, probably syntonin. The only process which is known to produce albumin absolutely free from ash is that of Graham (Liebig's *Ann.* 121, 61); see also Aronstein (*Archiv Physiol.* 8, 1874, 75). The albumin to be purified is freed from membranes by agitation and filtration; the albumin may be diluted with an equal volume of water, or it may be filtered without dilution, by means of the pressure-filter described in my researches on the brain. The albumin thus prepared may be acidulated with dilute acetic acid, if it is desired to expedite the following processes; but when it is desired to separate pure plasmin (paraglobulin) it is perhaps advisable to avoid the use of acetic acid, as it might possibly transform some of the albumin into syntonin. It has, however, not been proved that such a transformation does actually take place under these circumstances.

The albumin, whether acidulated, neutral, or feebly alkaline is now placed into a cell of a size and shape found by experience to be convenient for dialysis. This cell is made, as far as its active surface is concerned, of parchment paper prepared by the process of Gaine. Great care must be taken to procure actual parchment paper of the best quality, and to avoid the counterfeited paper made by means of gelatin and alum. The layer of albumin in the cell should be thin, and the water into which it is placed should be renewed two or three times a day. The apparatus should stand in a room the temperature of which is about 10° to 12°. Four to five days of dialytic action are sufficient to free the albumin from all salts.

The albumin to be placed into the dialysing cell should first of all be measured, and should again be measured after the dialysing is completed, in order to ascertain the quantity of water which passes into the cell and becomes mixed with the albumin. It is further advisable to keep a measured quantity of the same albumin as that which is placed in the dialyser in an unmixed state, and allow it to stand by the side of the dialysing albumin, in order to be able to compare the reactions of the original impure albumin from time to time with the specimen which is being purified by diffusion.

If it is desired to obviate the difference in concentration between the original and the dialysed albumin, the latter may be concentrated to its original volume over sulphuric acid *in vacuo*, or the former may be diluted with water to the bulk of the dialysed specimen. The amount of the increase in volume by complete dialysis amounts to about one-half the original volume.

Should the liquid become undesirably voluminous, and should diffusion proceed but slowly, it is sometimes advisable to remove the albumin from the dialyser, filter it from any plasmin which may be deposited, and evaporate it to a smaller bulk *in vacuo* over sulphuric acid. The concentrated liquid, on being returned to the dialyser, parts with its salts much quicker.

*The diffusate or dialysate.*—The diffusates are united and evaporated to the volume of the albumin originally placed in the cell; during this evaporation small quantities of albumin, which pass into the diffusate, are coagulated, and have to be removed. After this the diffusate is free from all albuminous matter. It contains sodic chloride, and sulphate, sodic and potassic phosphate and carbonate; but even when strongly oversaturated with ammonia, it deposits no earthy phosphates.

The diffusate contains peculiar nitrogenous matters in solution which have not yet been investigated. On evaporation to dryness it leaves a yellowish-brown residue, which burns with a strong odour of horn. When the matter is completely calcined, it leaves an ash, of which a part is soluble, another part insoluble in water. The latter is soluble in hydrochloric acid, and consists of phosphates of calcium and magnesium with excess of lime (and magnesia?).

It is therefore clear that the solution of the earthy salts in the albumin (serum or egg) is caused by one or several of the nitrogenous matters which also accompany these salts into the diffusate. When the dialysis is complete the whole of the soluble and insoluble salts have passed into the diffusate, and the albumin in the cell is completely devoid of them; this proves that the phosphates of earths and the earths cannot be, as is sometimes assumed, in combination with the albumin; they are however in a soluble combination with nitrogenous matters, which are not albuminous.

*Deposition of the plasmin (paraglobulin) during dialysis.* Soon after the albumin has been put into the dialysing cell it begins to become turbid, and at the same time the presence of alkaline salts in the diffusate can be proved by reagents. If the albumin be filtered every 24 hours, and the water surrounding the cell be renewed, it will be perceived that each time a new turbidity is produced in the albumin, and the diffusate contains salts anew. But the quantities of deposit in the albumin and of salts in the diffusate diminish at a certain rate, so that in equal times more deposit and salts separate from the albumin at the beginning of the experiment than towards the conclusion. At last there comes a period in the experiment, at which the albumin remains clear, and the diffusate contains no trace of salt. The solution in the cell then contains the albumin in a pure state; the diffusates contain all the soluble salts, and the insoluble salts dissolved by the peculiar nitrogenous matters, and all the plasmin has been collected on filters.

The plasmin consequently was dissolved in the original (serum or egg) albumin, by means of the substances which pass into the diffusate. If the plasmin collected on filters, is shaken up with a little water, and if then the diffusate concentrated to the volume of the original albumin solution is added to it, it redissolves immediately and completely.

This plasmin is identical with the plasmin which can be precipitated from serum and egg albumin by dilution with much water, acidification and filtration; when serum or egg albumin thus treated and again concentrated to its primitive volume, is subjected to dialysis, it does not any longer form the deposits above described. Inversely, when the albumin, freed



from all salts by dialysis and filtered from the insoluble deposit which forms during that process, is diluted with much water, and very slightly acidified, no deposit takes place. It is therefore almost certain that both precipitates are made up of one and the same substance, namely plasmin. It is insoluble in water, easily soluble in dilute acids and alkalies, in carbonates of alkalies, and in neutral salts of alkalies. It also can be utilised for the production of fibrin, a circumstance to which it owes its name.

*Characters of the albumin freed from salts &c. by dialysis, and from plasmin by filtration.*—Evaporated to dryness and burned with all necessary precautions in platinum vessels it leaves not a trace of ash or residue of any kind. Diluted with from 8 to 10 parts of water, and boiled in that state, or if it is alkaline or neutral, previously acidified by a little acetic acid and boiled, it remains perfectly clear. It is not changed in any manner by the addition of strong alcohol, even of absolute alcohol up to twenty volumes. If the albumin is mixed again with the diffusate, reduced to the concentration which corresponds to its share of the bulk of the original albumin, a solution is produced which has all the properties of the original albumin, inasmuch as it curdles on boiling and by the addition of alcohol. The process of diffusion, though extending over several days, has therefore not produced any material changes in the properties of the albumin itself; while the pure albumin of serum and eggs is not coagulable by heat or alcohol, the albumin containing salts is so coagulable. It is therefore clear that the coagulability of the natural solution of albumin depends essentially upon the presence of crystalloid bodies.

When the part of the ash of the diffusate soluble in water is mixed with pure albumin, it also regains its coagulability by heat and alcohol. The addition of only 0.25 grm. of sodic chloride to 100 c.c. of pure albumin solution restores its coagulability. Potassic chloride, and iodide, sodic iodide, magnesian sulphate, and ammoniac carbonate have the same effect. The coagulability of natural solutions of albumin therefore depends on the presence in them of soluble salts.

*Quantities of salts necessary for the production of the coagulable state.*—If during the progress of the dialysis small

samples of the albumin are taken from the dialyser from time to time, it will be found that the precipitates produced by alcohol or boiling are constantly decreasing in bulk and importance; towards the end of the dialysis only turbidities are observed on the application of the reagents, a little later on opalescence constitutes the result, and ultimately the albumin remains quite clear, indicating the complete removal of the salts. This proves that for the coagulation of a certain amount of albumin a certain minimum amount of salt is required to be present. It is probable that this minimum has as determinants the amount of water in which it is contained, as well as the amount of albumin which is dissolved in it.

When to portions of a solution of albumin freed from salts by dialysis very small but increasing quantities of sodic chloride are added, a series of liquids are obtained which, on application of heat or of alcohol, present all degrees of coagulation, from a mere opalescence to the complete precipitation of all albumin. The amount of sodic chloride necessary to effect a complete coagulation of the albumin from either white of egg or blood-serum is much smaller than the amount of salt usually contained in these natural fluids; or these fluids contain more salt than is required to produce coagulation by heat or alcohol.

If to pure albumin very small quantities of salt are added, a portion only of the albumin can be precipitated, and this part stands in a certain relation to the amount of salt present. This relation can be ascertained by adding known quantities of salt solution to known quantities of pure albumin of given concentration, and boiling or adding alcohol. The precipitate produced is filtered and weighed, and referred to the sodic chloride employed; the filtrate is then completely precipitated with the aid of excess of sodic chloride; the sum of the two precipitates gives the total albumin. The effects of such small quantities of salt as lead to the production of only an opalescence or a turbidity cannot be measured by weighing the precipitates, which would probably run through any filter, but can only be estimated by measuring the amount of most dilute salt solution which was required to engender their production, and calculating from this and the data given by more concentrated solution their probable amount. One hundred c.c. blood-serum, which

during dialysis had lost almost the whole of its salts and had gained 21 c.c. of water, were subjected to the following tests by Aronstein. The tests were compared with similar tests made upon a portion of the same blood-serum which had not been subjected to dialysis but had been diluted with water in the proportion of 21 c.c. of the latter to 100 of the serum. The amount of alcohol added in each case was sixteen times the volume of the liquid to be precipitated. The precipitates were collected on filters and dried, and weighed with the usual precautions. The following numbers refer to 100 c.c. of the undiluted serum.

One hundred c.c. of natural serum gave 6.521 grms. coagulated albumin.

One hundred c.c. of serum freed from salt by dialysis gave on addition of 0.030 grms. NaCl 2.423 grms. coagulated albumin; on addition of 0.080 grms. NaCl 3.831 grms. coagulated albumin; on addition of 0.160 grms. NaCl 4.859 grms. coagulated albumin.

The serum free from salt gave a lesser maximum of precipitate than natural serum, because the process of dialysis removes small quantities of albumin, which pass into the diffusates, or precipitates matters like plasmin, which are precipitated from serum together with the albumin and weighed with it. Aronstein believes that extractive matters also might be included in the serum precipitates; of this however there is no direct proof.

The data further show that as the amount of salt added to the solution of albumin rises, so the quantity of albumin precipitated from it by alcohol rises; further that the whole of the albumin is precipitated from a solution of the strength of the one operated upon (which contained nearly five per cent. of albumin) when the amount of salt contained in it amounts to 0.160 per cent. Ordinary blood-serum therefore contains about four times as much soluble salt as is necessary to ensure its coagulation by heat or alcohol, namely about 0.6 per cent.

These data prove that the statements of Kühne and Hoppe-Seyler, according to which albumin free from salt could not be obtained by dialysis are not well founded, and that their proceedings must have been vitiated by either faulty manipulation or bad materials.

*Relation between the quantity of salt and the strength of the alcohol required for precipitation of albumin.*—The amount of salt and the strength of alcohol stand in an inverse proportion to each other. Two equal volumes of a pure solution of albumin were mixed the one with 16 vols. of spirit of 45 per cent. strength, the other with 16 vols. of alcohol of 90 per cent. strength. Both liquids remained perfectly clear. To each was now added a very dilute solution of sodic chloride in very small quantities from a very finely divided burette admitting of the reading of a hundredth part of a cubic centimetre, until the albumin began to be precipitated. The mixture with the spirit of 45 per cent. required 0.860 per cent. sodic chloride to precipitate the albumin; that with the 90 per cent. spirit required only 0.017 per cent. sodic chloride, or only one fiftieth part of that which the more dilute spirit made requisite. The action of the salt in alcoholic solution is therefore not uniform with the percentage, but increases manifold with the strength of the alcohol.

A solution of albumin freed from salts by dialysis which will remain perfectly clear with 16 vols. of spirit of 90 per cent. will yet sometimes give a turbidity or opalescence on the addition of 16 vols. of absolute alcohol. Such albumin on incineration gives no ash and no reaction for chlorine in the platinum vessel used for the combustion. The opalescence is therefore the only indication of the presence of salts, and is a more delicate reaction for chlorine than the silver test. This shows the necessity of working in these experiments with absolutely pure reagents and vessels. The mere presence of a few drops of ordinary well water, or some atmospheric dust containing as it always does chloride of sodium, is capable of vitiating the experiment, and to produce precipitates where if they had been excluded there would not have been any.

*Incipient decomposition restores coagulability of solutions of pure albumin.*—When a solution of pure albumin, obtained by dialysis, is allowed to stand for some days, it begins to decompose, and then is again precipitated by heat as well as alcohol. The precipitability begins with a feeble opalescence, and gradually increases so much that all the albumin nearly can be coagulated. This return of the original properties is due to the formation, by decomposition of some part of the albumin,



of salts, particularly ammoniacal salts. Aronstein tested the carbonate of ammonium, and found it as active in engendering coagulability as sodic chloride. It is therefore necessary to keep all dialysing fluids at a very low temperature, below  $12^{\circ}$  at least, and as near zero as circumstances permit. The present experiment however also shows, that an albumin which gives, after dialysis, the reactions of pure albumin, cannot be decomposed.

*Difference of serum, and egg-albumin in their behaviour towards ether.*—Serum and egg-albumin show the same reactions towards all bodies known to react with them, except ether; from white of egg the albumin is precipitated by ether, from serum it is not precipitated by this agent. But when the albumins are free from salts, they show the opposite bearing; the pure egg-albumin is not precipitated by ether, while the pure serum-albumin is precipitated. If to the pure solutions, a little salt is added, the properties of the natural solutions are restored. With egg-albumin, the presence of salt is a condition of the coagulability by ether, while as regards serum-albumin, the presence of salts prevents coagulation by heat.

From the foregoing it follows that the views put forward by Scherer, Denis (*Mémoire sur le sang*, 187), and Eichwald (*Würzb. Med. Zeitschr.* 5, 318), (probably under the influence of Scherer) viz. that egg-albumin and animal albuminous substances generally are in themselves insoluble in water, and become soluble only after combination with salts or alkalies, is not tenable as regards the great bulk of the substance termed albumin, but is correct only as regards a small portion of the albuminous matter occurring in animal liquids, namely the plasmin (paraglobulin, or serum-casein), and the fibrinogenous matter so called.

*Quantation of albumin in animal liquids, with the aid of dialysis, according to Heynsius* (*Archiv Physiol.* 10, 1875, 239). Heynsius objects to the use of nitric acid for the precipitation of albumin; firstly, because precipitates of uric acid might simulate albumin; secondly, because in liquids containing a small proportion of salts nitric acid redissolves a small quantity of albumin, and forms acid-albumin. He formerly recommended the precipitation of albumin by boiling in presence of

acetic acid and sodic chloride (1870; he does not claim the method as having been new then), but he now finds the method, though still suitable for removing all albumin out of animal liquids, unsuitable for its quantation, as the washing required dissolves much curdled albumin and carries it into the filtrates.

In some experiments on partially dialysed white of egg, Heynsius found, that the addition to 50 c.c. of the liquid of 2 c.c. of ordinary acetic acid and quantities of sodic chloride varying from 32 per cent. (saturation) to 4 per cent., and boiling, all albumin was precipitated, so that the filtrates did not contain any; from 2 to 0.1 per cent. of sodic chloride enabled some albumin to remain in solution. Heynsius then criticises the method of Scherer, namely addition of acetic acid to the animal liquid in such quantity that the mixture after boiling shall yet have an acid reaction, a process in which he alleges that acid-albumin is formed; and says that it gives results which are too low, because a part of the albumin remains dissolved—in two eventualities, either when (and because) the acid was in excess relatively to the salt, or the salt was in excess relatively to the acid. The statement of Heynsius on this point is by no means easily intelligible.

Liborius (*Deutsch. Archiv klin. Med.* 10, 1872, 320) had examined the bearing of albuminous solutions, during boiling, in two series of experiments; one in which the quantity of acetic acid was stationary, while that of the sodic chloride varied; and another in which the amount of sodic chloride was stationary, while that of the acetic acid varied, and had found that in both series the amount of albumin obtained was less than that actually contained in the liquid.

Heynsius made a number of similar experiments on white of egg and serum, and came to the same result. A minimum of sodic chloride and of acetic acid gave the largest amount of albumin. But the amount obtained never was the whole amount present, as the washing water always dissolved albumin, which could be identified. He therefore declared this method (of Scherer) unsuitable for the quantation of albumin. The so-called method of Berzelius, consisting in evaporating the albuminous liquid, mixed with a little acetic acid, to dryness, and extracting the residue with alcohol, also gives results which

do not express the whole of the albumin present, some of it going into the extracts.

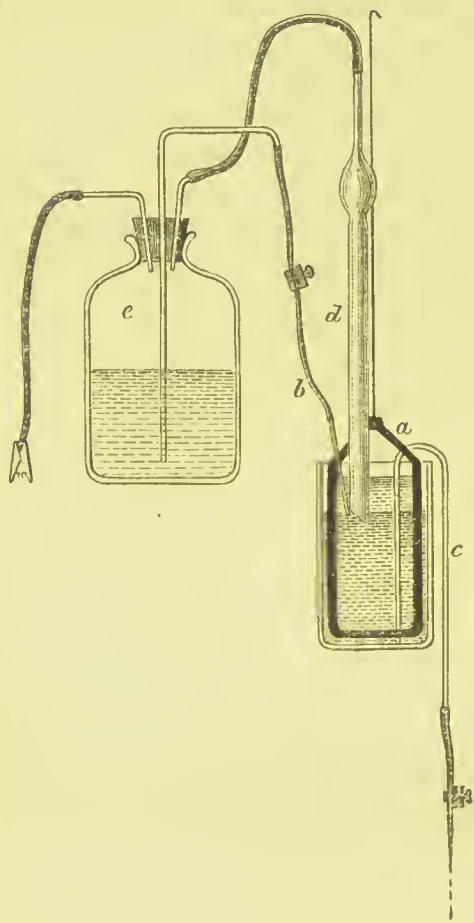
Liborius and Heynsius now come to the result, that the albumin is probably most accurately estimated by precipitation with alcohol, after its solution has been carefully neutralised, But in this case a considerable amount of ash remains in the albumin (from 10 to 20 per cent. of the entire ash), which has to be found by incineration and deducted.

To avoid this drawback, Heynsius subjects the albuminous solution in which the albumin is to be estimated, to dialysis, and evaporating the solution, after removal of crystalloids, to dryness, assumes this residue, less 2 per cent. of ash, supposed not to be removable by dialysis, as the amount of albumin in the original fluid. It deserves to be mentioned that Heynsius employed parchment paper, which allowed so little albumin to pass into the diffusate, that it did not affect even the second decimal of the quantities dealt with in his experiments.

If albumin had to be determined in urine by this method, salts might be deposited and escape dialysis. In such a case it might be useful to dialyse the liquid successively, first in the alkaline, next in the acid condition.

From an inspection of the data adduced by Heynsius in proof of the advantages of his method, it appears that they are literally very small. If it is considered that the amount of ash retained in the dialysed albumin is found to vary with every specimen of dialysing paper employed, and that notwithstanding this experience, a uniform 2 per cent. of ash is to be deducted, it cannot be admitted that the method can be employed by anybody else besides Heynsius, unless they procured paper which permits no albumin to pass into the diffusate, and unless they determine the average amount of ash (if any) retained by the dialysed albumin. It is more than doubtful whether this amount will be a law of nature so fixed as to enable the observer to dispense with a quantation in each case. And if such be necessary, then it would be more practical to estimate the ash in the albumin precipitated by alcohol (10 to 20 per cent. of the entire ash) than the much smaller quantity contained in the dialysed albumin, and to save the trouble and time expended upon the dialysis.

D. Huizinga's dialyser (*Archiv Physiol.* 11, 1875, 392) consists of a frame of vulcanite (hardened caoutchouc) 5 millimetres thick, 1 centimetre broad, and enclosing an area of about 70 square centimetres. The lower or square part of this is covered on both sides with parchment paper. The papers are



cut a little smaller than the external margin of the frame, as they expand when they are moistened. The vulcanite must be made rough with sand or emery on the outer sides, where the paper is intended to be fixed. The paper in the wet state is fixed upon the frame by means of chromate glue. To produce the latter 10 grms. of dry gelatin are dissolved in 50 grms. of water, and to this solution 0.5 grm. potassic bichromate dissolved in 10 grms. of water is added. This glue must be preserved in the dark, as it is made insoluble by light. It must also be made fresh from time to time, as repeated heating has a tendency to make it lumpy.

The vulcanite frame to which two pieces of parchment paper have been pasted one on each side by means of this glue, is exposed to diffuse daylight, whereby the glue becomes insoluble; the parchment paper must remain a little moist, to prevent its fissuring. The cell thus made is tested with water, and if it is tight, is freed from the excess of chromate by immersion in water. It is now ready for use with albumin or other matters. It is placed in a flat glass cell which is



only a little larger than itself, and filled with albumin, prepared as above described, to about two-thirds of its height. The space between the glass and the dialysing cell is filled with distilled water. This latter is frequently renewed by an automatic arrangement (such as is described on p. 127 of my researches on the brain, *Rep., New Series*, N<sup>r</sup> III.), termed a perpetual syphon, while the water which has performed dialysis and is charged with products is drawn off at intervals and in a larger stream, or constantly and in a smaller current, by a second syphon, delivering into a receptacle for waste, and drawing the waste from the bottom of the glass cell. The principal advantages are that the thin layer of liquid is dialysed on two sides, and therefore in a lesser time than by the tambourine-dialyser; and further that any deposit which forms in the liquid sinks to the bottom of the frame, and has little chance of obstructing the paper, an accident which not rarely happens with the horizontal paper, particularly in the case of albumin, from which plasmin is always precipitated during dialysis.

The albumin to be placed into this cell should be carefully neutralised; acid albumin dialyses very slowly, as already observed by Graham. 80 c.c. of albumin in the cell should be acted upon by a litre of water per hour for 24 hours; in that case the albumin does not coagulate any more either by heat or alcohol when diluted with the fourfold volume of water; after 36 hours the albumin ceases to react with silver nitrate or mercuric chloride. After 48 hours the albumin is no longer changed by the dialytic process. It has increased in bulk but little, is turbid by flakes, but passes through a filter of Swedish paper, freed from ash by treatment with acids etc., quickly and clear.

A portion of albumin thus treated is evaporated in a weighed platinum dish on the water-bath, the weight of the residue is determined, and the residue is charred. On extraction of the char with hot water, it is found that it does not contain a trace of soluble salt. The water, though perhaps containing a trace of chlorine, leaves no residue and no weighable matter in the platinum dish in which it has been evaporated. The charcoal is now burned white, and leaves a small quantity of incombustible matter containing calcium, iron, a trace of magnesium,

and phosphoric acid. In Huizinga's quantation the amount of ash thus obtained varied from 0.35 to 0.56 per cent. of the albumin burnt; and the amount of dry dialysed albumin burnt varied between 0.310 grms. and 1.1376 grms.

The dialysed solutions of albumin, if undiluted, give a precipitate with silver nitrate and with mercuric chloride. When the albumin is diluted with four volumes of water, these reagents no longer produce a precipitate by themselves: but when to the mixture with silver nitrate a drop of nitric acid is added, a considerable flaky precipitate is at once produced.

*Bearing of dialysed albumin solution with acetic acid.*—Schmidt found that the addition of very dilute acetic acid to dialysed albumin restored its coagulability by heat, but a more considerable addition of acetic acid produced again incoagulability. When Huizinga added to 5 c.c. of a solution of albumin in 4 water, containing therefore 1 c.c. of original albumin, —1 c.c. of acetic acid of one-fiftieth per cent. strength (therefore equal to 0.2 milligrams  $C_2H_4O_2$ ), the mixture coagulated strongly on boiling; but when acetic acid of one-hundredth per cent. strength equal to 0.1 milligram  $C_2H_4O_2$  was taken, only an opalescence and no precipitate was produced.

It was easy to suppose that the acid in the first case (0.2 milligr.  $C_2H_4O_2$ ) had been sufficient to decompose an assumed albuminate, while the 0.1 milligr. in the second case might have been insufficient for that purpose. If this explanation was correct 5 c.c. of a solution of albumin diluted to ten times its bulk, therefore containing 0.5 c.c. of original albumin, should have been coagulated by boiling after addition of 1 c.c. of acetic acid of one-hundredth per cent. strength. But this was not the case; no coagulation took place; while 1 c.c. of the one-fiftieth per cent. acetic acid produced coagulation. On diluting albumin twentyfold the same result was obtained. 5 c.c. of this albumin mixed with 1 c.c. of one-fiftieth per cent. acetic acid coagulated in flakes on boiling; while 5 c.c. of the same solution mixed with 1 c.c. of  $\frac{1}{100}$  per cent. acetic acid only opalesced on heating. Only when the dialysed albumin was diluted to forty times its bulk did boiling after the addition of 1 c.c. of  $\frac{1}{100}$  per cent. acetic acid to 5 c.c. of the solution produce a precipitate.

There is therefore no direct proportionality between the quantity of albumin and the quantity of acetic acid which makes the albumin coagulable, and therefore it is not probable that the acid acts by displacing a base from its combination with albumin.

*Bearing of dialysed albumin with increasing quantities of acetic acid.*—When to 5 c.c. of a dialysed solution of albumin diluted to its tenfold bulk 1 c.c. of acetic acid of 0.02 per cent. strength was added, and the liquid was boiled, flaky coagulation took place. With the same quantity of albumin and 1 c.c. of acetic acid of 0.04 per cent. strength, the coagulation on boiling was imperfect. Upon the same quantity of albumin, the same volume of acetic acid, increasing in strength by 0.02 with each experiment acted so that only opalescence was produced, which decreased in density as the acid increased in strength; 5 c.c. of the albumin solution with 1 c.c. of acetic acid of 0.25 per cent. strength remained clear on boiling, and double the amount of acid also left the liquid clear. In order therefore to retain the albumin in solution on boiling (after having destroyed its solubility on boiling by the addition of most minute quantities of acetic acid), it is necessary to add so much acetic acid that its amount in the mixture shall be at least  $\frac{1}{2\frac{1}{4}}$  per cent. of the mixture. When the dialysed albumin diluted to ten times its bulk has become opalescent by boiling, much larger quantities of acetic acid can be added to it, without any precipitate being produced by boiling. The fluid however which has become opalescent by boiling, gives with silver nitrate a flocculent precipitate.

Larger quantities of acetic acid keep the albumin in solution on boiling, so that the fluid remains clear. But the amounts of acid necessary vary with the concentration of the albumin. We have seen before that in order to cause 5 c.c. of the dialysed albumin diluted to its tenfold bulk to remain clear on boiling, the addition of 1 c.c. of acetic acid of  $\frac{1}{4}$  per cent. strength is requisite. From this it might be supposed, that in order to produce the same effect upon a solution of albumin of double the concentration, the addition of 1 c.c. of acetic acid of  $\frac{1}{2}$  per cent. strength would be sufficient. This, however, is not the case; 5 c.c. of the albumin solution diluted to fivefold its bulk,

requires, in order to remain clear on boiling, the addition of 1 c.c. of acetic acid of 1 per cent. strength.

With rising concentrations of the albumin, the quantity of acetic acid necessary to keep it in solution on boiling also increases, but not in an equal proportion. For in order to remain clear on boiling, 5 c.c. of a solution of albumin diluted to fourfold bulk require 1 c.c. of acetic acid of 2 per cent. strength; 5 c.c. of a solution diluted to twofold bulk, require 1 c.c. of acid of 20 per cent. strength. The undiluted dialysed albumin solution requires upon 5 c.c., 2 c.c. of concentrated acetic acid, to retain all the albumin in solution on boiling. The quantity of acid necessary to retain the albumin in solution, rises much quicker than the amount of albumin to be retained.

The albumin solution as it leaves the dialyser of Huizinga, contains from  $1\frac{1}{2}$  to  $3\frac{1}{2}$  per cent. of albumin, when the albumin on entering the dialyser amounted to about 5 per cent. of the solution.

*Taste of dialysed albumin.*—The taste of a dialysed solution of albumin of from 2 to 3 per cent. strength is clearly sweet; the taste is an aftertaste, and resembles that of liquorice root. When the dialysed albumin has been dried and heated, it is perfectly tasteless.

A. Winogradoff (*Archiv Physiol.* 11, 1875, 605) also subjected white of egg to dialysis, on the so-called parchment paper used by A. Schmidt; the dialysers were 18 centimetres in diameter. The albumin was always diluted with three times its volume of water, and filtered through paper, but not neutralised. When the outer water did not any longer contain chlorides the process was interrupted: the albumin had the properties described by Aronstein, but possessed an alkaline reaction, colouring reddened tincture of litmus blue;  $\frac{1}{10}$  normal acetic acid, that is to say a solution of 60 grms. of crystallisable acetic acid in 940 grms. of water, was further diluted with two volumes of water (so as to contain 60 grms. of acid and 2940 grms. of water), and used for neutralising the albumin; the acid having been added drop by drop until the albumin had assumed a violet-red colour, the solution was now heated, when all the albumin was precipitated; the filtrate was so free from albumin that when it was mixed with an equal volume of solution of salt



and some acetic acid, it remained perfectly clear, and when evaporated by itself and the residue burned only a slight charring was observed. This experiment showed therefore that a solution of albumin which had lost its coagulability by heat and alcohol in consequence of its having undergone dialysis, recovered its coagulability by an addition of acetic acid.

Winogradoff now made some experiments to determine the amount of ash, if any, which solutions of albumin subjected to dialysis, might retain. Each dialysis was made with 30 c.c. of egg albumin, to which 90 c.c. of water had been added. The dialysis was continued until the diffusate was free from chlorine, the solution of albumin on the dialyser was then measured, treated with acetic acid in the manner described, and coagulated on the water-bath. The albumin was collected on weighed filters, dried and weighed, and then incinerated. The filtrate from the coagulated albumin and the washing water were also evaporated and incinerated, the diffusates were treated in the same manner.

The following tables show the results of the experiments as obtained by means of two different kinds of dialysing paper, one sized so-called English, as used by A. Schmidt, another German, thicker than the English, of uncertain quality, as used by Heynsius.

I have divided the table of Winogradoff into two tables, so as to keep apart statements referring to absolute quantities operated upon, and figures derived from absolute quantities by calculation referred to percentages.

	Experiment I.		Experiment II.		Experiment III.	
	Sized	Uncertain	Sized	Uncertain	Sized	Uncertain
120 c.c. of dilute albumin, containing 30 c.c. of white of egg, rose to	218 c.c.	140 c.c.	240 c.c.	138 c.c.	230 c.c.	154 c.c.

It is at once evident that the amount of water which passed into the albumin on the dialyser, and inversely the amount of albumin which passed from the dialyser into the diffusate, were much greater when so-called English sized paper was used, than when German paper of uncertain quality was employed.

Winogradoff does not describe the nature of the German paper used by him, but of the so-called English paper he says that it was the same as that used by A. Schmidt, and actually forwarded to him by this author. The latter had in the interval ascertained that this so-called English parchment paper, though obtained from London, and bearing the watermark of a London firm, was in reality made in Germany, and was not parchment paper made by the process of Gaine, such as had been used by Graham, but a paper to which hardness and transparency had been given by impregnation with gelatin and alum; the dialysis was therefore in reality carried on through a septum of gelatin and alum, or rather through two septa of gelatin and alum one on each side of a piece of common paper.

The following table will after this explanation be much more instructive than it could have been without it. For it will show that the size and alum paper besides passing much more albumin into the diffusate than the other paper, diffused a lesser quantity of the salts of the albumin into the dialysate, and left more salt in the albumin on the dialyser, so that both the curdled albumin and its mother liquor left more ash than the corresponding preparation obtained with the other (German) paper. Neither of the papers gave the results obtained by Graham, possibly because the nondescript German paper also was not

	Experiment I.		Experiment II.		Experiment III.	
	Sized	Uncertain	Sized	Uncertain	Sized	Uncertain
Albumin in 100 parts of white of egg . . .	Not known		10.49		10.83	
Albumin in solution after dialysis . .	7.87	9.87	7.26	8.69	6.34	9.60
Ash in diffusate . . .	0.467	0.548	0.591	0.625	0.614	0.636
Ash in curdled albumin . .	0.0186	0.0296	0.0226	0.0240	0.0233	0.0206
Ash in filtrate from curdled albumin . .	0.1671	0.1493	0.1446	0.1120	0.1846	0.1463
Sum of ash in dialysed albumin solution	0.1857	0.1789	0.1672	0.1360	0.2079	0.1669

paper prepared by the process of Gaine, but some imperfect imitation, though a better dialyser than the thinner size and alum paper.

The foregoing table gives the results of the dialysis in three experiments made each with the two kinds of paper. They are calculated upon 100 parts of the original white of egg, the dilution by water added before dialysis, and by water transferred during dialysis, being deducted.

The salts consisted of carbonates and chlorides of alkalies, and of phosphates of earths. The dialysates of the sized paper moreover gave sulphates.

The inequality in the action of the two kinds of paper is not only seen in the first table but in almost all the items of the foregoing second table. The second (uncertain) paper removes much less albumin into the diffusate than the sized paper; it removes, on the contrary, a much larger quantity of salt out of the albumin, a quantity which (considered as work performed by the dialyser) would appear much greater than in the table, if the salts, which must have passed *with* the albumin to which they were potentially related, are deducted from the ash in the diffusate, not accompanied, so to say, by the albumin previously related to it.

It is conceivable that as of two indifferent dialysing papers one removed more, the other less of salts out of the white of egg to be purified, a paper of the quality such as Graham employed made by the process of Gaine, and not impregnated with any size or chemical ingredient whatever, may produce the effects described by Graham. Until that point is decided by incontrovertible experiment, a final view concerning the nature of pure albumin solutions cannot perhaps be formed. But how little the experiments hitherto made are comparable is not only evident from their study, but from even slight experimental epicrisis. While writing these lines I dialysed the white of three eggs through thin good parchment paper, until it was incoagulable by heat and alcohol. *At no stage of the dialysis was there any albumin whatever in the diffusate.*

Winogradoff made three further experiments, but without specifying the paper used as dialyser, and without comparing the

two papers with each other. We will term the experiments N<sup>rs</sup>. IV. V. and VI.

*Exp. IV.*—Twenty-five c.c. of albumin were mixed with 25 c.c. of water and dialysed in a bell-shaped dialyser suspended in water for forty-six hours, during which the water was renewed eleven times. At the conclusion of the dialysis the volume of the 50 c.c. of liquid on the dialyser had increased to 224 c.c. It did not curdle on being boiled, but became slightly turbid. Considered as condensed to the original 25 c.c. and multiplied by four, so as to represent one hundred parts of albumin changed only by the loss of salts (and albumin), the fluid gave on analysis the following data: Albumin 10.98 per cent.; ash (earthy phosphates) in curdled albumin 0.0236 per cent.; ash in filtrate from coagulum (being chloride, phosphate and sulphate) 0.1224 per cent., or total ash of the dialysed liquid 0.1451 per cent. (Winogradoff says that these data are ‘= 1.32 per cent. ash in the albumin, and of this 0.215 per cent. earthy phosphates.’ Here albumin means the imaginary dry residue of the dialysate. I have been obliged frequently to surmise the cohesion of data and calculations in his paper, putting on them the best constructions possible, a trouble which the author might with advantage to himself have spared to his readers.)

*Exp. V.*—The fresh white of egg contained 12.31 per cent. albumin; its coagulum contained salts 0.0333 per cent. of the white of egg, and the filtrate from this 0.6626 per cent. Thirty c.c. of this white of egg were diluted with 30 c.c. of water and dialysed during 62 hours in water which was thirteen times renewed. The volume of the albumin had risen to 370 c.c. or more than six times the initial volume.

Considered as condensed to the original 30 c.c. and multiplied so as to represent one hundred parts of white of egg changed only by the loss of salts (and of some albumin), the fluid gave on analysis the following data: albumin 8.96 per cent.; earthy phosphates in the coagulum 0.0176 per cent.; earthy phosphates in the filtrate 0.0986 per cent.; total ash 0.1162. (Here Winogradoff also says that this was equal to ‘1.29 per cent. ash in the albumin, and of this 0.16 per cent. earthy phosphates;’ from which we may conclude that by albumin he means here also the imaginary dry residue of the dialysate.



*Exp. VI.*—This dialysis was made with 30 c.c. of white of egg diluted with 30 c.c. of water, in a bell-shaped dialyser suspended in water which was kept constantly fresh by a current of 250 c.c. per hour, increased after 36 hours to half a litre per hour (time during which the latter treatment lasted not stated). At the conclusion of the dialysis the liquid in the cell measured 175 c.c.; it contained a considerable deposit of paraglobulin, and had a feeble alkaline reaction. Addition of acetic acid and boiling curdled all the albumin. The analyses of the liquid calculated as in the previous two experiments gave albumin only 2.37 per cent.; salts in the coagulum 0.0020 per cent.; salts in the filtrate 0.0173 per cent.; total salts 0.0193 per cent; or the dried residue of the dialysed liquid contained 0.81 per cent. of ash, and of this 0.084 per cent. were earthy phosphates. Although in this liquid the salts had been more diminished than in any previous one, it coagulated on the application of heat alone, without any addition of acid.

The enormous loss of albumin shows that the dialyser was very bad in one respect, although it effected a great removal of crystalloids. The coagulability Winogradoff refers to a return of this faculty (which he considers, so it seems from the context, had been lost in the course of the earlier period of the dialysis) but he does not believe it to be due to beginning putrefaction, as Aronstein does, who also assumes the formation of some ammoniac carbonate, and ascribes to this the return of coagulability by heat.

This return of the coagulability by heat and alcohol in albumin freed from this liability by dialysis I have also observed, after filtering it through paper not previously expressly freed from salts by acids and water etc. I have however not yet proved that the renewed coagulability is due to the admixture of salts from this source. It is possible that the albumin, though not smelling in the least of decomposition, may nevertheless be altered, as by *time* it alters in almost all known solutions.

*Properties of the natural white of egg.*—It can be dried, when in thin layers, quickly in a current of air, and then forms

a fissured, hard, brittle, white or yellowish translucent mass, which has neither taste nor smell. It is again soluble in water, but leaves a slight insoluble residue. With four parts of cold water it forms a mass resembling fresh albumin, and with more water a slimy solution. This is perhaps referable to the oonin, the mucus-like substance removable by freezing, which swells in contact with water to a large bulk, and can only with difficulty be filtered from the albumin.

*Polarisation.*—The clear solution of egg albumin rotates a ray of polarised light to the left, the specific limited rotation being  $(\alpha)^d = -35^\circ 5'$ ; the addition of hydrochloric acid increases this rotation to  $-37^\circ 7'$ ; strong caustic potash at first increases the rotation to  $-47^\circ$ , but reduces it later.

Danilewsky (*Krit. Zeitschr. f. Chem.* [2] IV., 12, 41) has stated that in hen's egg albumin there is contained an optically inactive albumin containing 2 per cent. of sulphur, by the splitting up of which albuminous substances are produced having a left-handed rotatory action on polarised light. In these bodies the sulphur is said to be combined in part directly and in part indirectly with oxygen; but an albumin may be obtained in which the sulphur exists only in direct combination with oxygen. Albumin containing 2 per cent. of sulphur, in being resolved into a—, b—, c— albumin containing 1.3, 1.1, 0.9 per cent. of sulphur, yields at the same time fatty acids, neurine and cholic acid as decomposition products. These last statements make it probable that Danilewsky had a specimen of albumin under his hands, which contained a phosphorised substance; the greatest doubt attaches to the alleged formation of cholic acid.

When a solution of white of egg free from membrane is allowed to drop into water, each drop becomes covered with a fine insoluble pellicle, and falls to the bottom undissolved. The gelatinous parts of the chalazæ show the same bearing, and remain as roundish swelled transparent particles surrounded by a white thin layer even on shaking with much water. The phenomenon is probably one of diffusion, by which insoluble albumin forms a crust round the drop, and prevents further diffusion of albumin, while favouring the diffusion of salts out of the drops of albumin into the water.

*Albumin not altered by frost.*—When albumin by itself or mixed with chloride of sodium, contained in a closed tube, is frozen in a mixture of solid carbonic acid and ether, it remains apparently unchanged and retains its fluidity after thawing and on exposure to the air. (Melsens.)

*Albumin altered by dilution and exposure to air.*—When common albumin mixed with an equal volume of water and strained is evaporated in the sun, or exposed in an open vessel for six days, it no longer coagulates on boiling, but at a certain degree of concentration solidifies to a translucent jelly which is soluble in water. The addition of very dilute acetic, formic, tartaric, or citric acid, by which the solution is not precipitated, restores the property of coagulation (Monnier, *Par. Chem. Soc. Bull.* 11, 470). According to this author a peculiar modification of albumin is thus produced.

*Coagulation of albumin.*—Ordinary natural albumin may be rendered insoluble *by motion*, and then assumes the appearance of fibres or membranes. This formation of membranes takes place also with albumin purified by Würtz's method. It occurs when albumin in drops is made to fall from a height, or dashed in spray against a surface; when dry or moist air, hydrogen, or carbonic acid, is passed through the solution, even under a layer of oil (in the case of precipitation by carbonic acid the product contains plasmin); when the solution is shaken for 24 hours in a closed flask, or when it is shaken in a vacuum. On evaporating *in vacuo* a solution of albumin saturated with sodic chloride, there remains a residue soluble in water, from which the insoluble membranes separate on passing carbonic acid through the solution, or on agitation (Melsens). See Harting's objections (Schmidt's *Jahrb.* 75, 148; *Jahresber.* 1852, 691), and Melsens' reply (*Instit.* 1857, 201; *Jahresber.* 1857, 531). Denis also obtained a large quantity of fibrillæ, insoluble in water and chloride of sodium, by prolonged agitation of albumin.

Another mode of rendering albumin insoluble is by heating its solution containing a sufficiency of a neutral salt, to from 63° to 75°. It is then converted into a white, slightly translucent, solid, somewhat elastic mass; this contains water in a colloid state, and dries up to a yellow horny substance, which

when immersed in water assumes the appearance of freshly coagulated albumin, but does not dissolve.

*Special features of coagulation by heat.*—The white of hen's eggs remains clear at 60°, becomes slightly turbid at 63°, begins to solidify at 65°, and becomes completely solid at 70° to 75° (Dumas and Prévost, *Ann. Chim.* 23, 52). Perfectly neutral solution of the white of hens' eggs (free from albuminate of soda (egg-casein) and globulin (or plasmin, etc.)), becomes turbid at 57°, forms a white pulp at 60°, and deposits large flocks at 62° to 64° (Lehmann, *Arch. f. Pathol.* 36, 115). The white of ducks' eggs begins to curdle at 63° (Chevreul, *Ann. Chim.* 19, 46; Gilb. 70, 379).

*Behaviour on heating of white of egg diluted with water.*—Fresh albumin, mixed with half part of water curdles completely when heated; with one part of water it remains slightly fluid; with ten parts no coagulum is produced but a milky turbidity which is perceptible even with 1000 parts of water (Bostock, *N. Gehl.* 4, 547; Schw. 29, 397). In these experiments the turbidity which ensues by mere dilution must be taken into account. The experiment of Bostock explains the error of Matthieu and Urbain, who ascribed the non-coagulation of diluted albumin to the abstraction of carbonic acid by the vacuum, to which they had subjected their diluted white of egg. On the behaviour of albumin diluted with water at 38°, see Arnold (Schmidt's *Jahrb.* 103, 1). A solution of Würtz's egg-albumin becomes turbid at 59°5, forms flocks at 63°, and solidifies at a somewhat higher temperature.

The coagulation by heat takes place also in vessels exhausted of air, without evolution of gas or change of volume. Fresh-laid eggs, when covered at once with oil, do not coagulate completely, but only become milky. The coagulation by heat is accompanied with the formation of hydrothion. Metallic silver is blackened by albumin when hot, but not when cold; coagulated albumin blackens lead acetate and also lead-paper held over it, after addition of acids (Gobley, *N. J. Pharm.* 18, 347). The coagulation is accompanied by elimination of alkali [ammonia (Eichwald, *Chem. Centr.* 1869, 568)], which combines with another portion of albumin to form albuminate (Scherer). Egg-albumin dried at a low temperature is not



coagulated, even at  $100^{\circ}$  [or above  $100^{\circ}$  (Brücke)] in the dry state (Al. Schmidt, Müller's *Arch.* 1862, 447). Commaille distinguishes boiled albumin as *pexin*. It dissolves with difficulty in slightly acidulated water. When its solution in caustic soda is precipitated by hydrochloric acid, and the precipitate is dissolved in water, a solution is formed, from which tetrachloride of platinum throws down a dark orange-yellow precipitate containing 7.80 per cent. of platinum (Commaille).

The coagulated albumin employed for the analyses given below was prepared either by boiling filtered white of egg and washing the coagulum with water and alcohol (Mulder, *Pogg.* 40, 271.—*J. pr. Chem.* 44, 489), or by precipitating filtered white of egg with alcohol, and washing with alcohol, water and ether. (Lassaigne, *Ann. Chim. Phys.* 20, 98; Dumas and Cahours, *N. Ann. Chim. Phys.* 6, 407; Theile, *Jenaer Med. Zeitschr.* 3, 147; *Jahresber.* 1867, 774). Rüling (*Ann. Pharm.* 58, 306) adds a few drops of hydrochloric acid before precipitating with alcohol.

*Elementary composition of coagulated and coagulable albumin. (Ash deducted in all cases.)*

	Gay-Lussac and Thénard	Mulder. Dried at $125^{\circ}$	Scherer. Dried at $100^{\circ}$	Dumas & Cahours. Dried at $140^{\circ}$
C	52.88	53.73	53.78	53.37
H	7.54	7.02	7.07	7.10
N	15.71	15.52	15.92	15.77
O	—	—	—	—
S	—	1.6	—	—
Ash	—	2.03	2.0	1.13

	Rüling		Würtz		Theile
	Dried at $100^{\circ}$	Dried at $140^{\circ}$	a	b	Dried at $130^{\circ}$
C	52.81	54.33	52.79	52.92	53.98
H	7.26	7.13	7.13	7.15	7.51
N	—	—	15.55	15.65	14.24
O	—	—	—	—	22.34
S	1.77	—	—	—	1.93
Ash	1.68	—	—	—	2.30

	Thiry	Schützenberger						
C	—	52.80	—	—	—	—	—	—
H	—	7.16	—	—	—	—	—	—
N	16.5	—	16.56	16.7	16.2	—	—	—
Ash	—	—	—	—	—	3.0	1.49	0.7

Würtz analysed the albumin prepared by his process in the soluble dried state (*a*) and in the coagulated state (*b*).

Theile's numbers are calculated from the analysis of albumin dried over calcic chloride, allowance being made for water expelled at 130°.

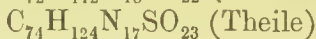
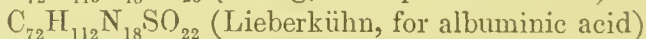
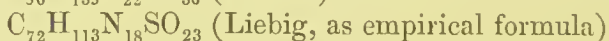
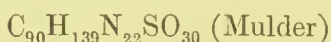
Mulder's albumin contained also 0.4 per cent. of phosphorus.

Verdeil (*Ann. Pharm.* 58, 317) found 2.109 per cent. sulphur.

Schwarzenbach (*Ann. Pharm.* 133, 185) found 1.85 to 2.2 per cent. of sulphur.

Schützenberger's analyses (*Ann. Chim. Phys.* 16, 1879, 305) were made upon albumin coagulated by heat, dried at 140° and exhausted with ether, reduced to an impalpable powder, and passed through a silk tammy. The amount of nitrogen was determined as nitrogen gas, and was found much higher than in the previous analyses, those of Thiry excepted (*Physiol. Zeitschr.* 1863).

*Older Formulae of albumin :*



In the second article, p. 43, it has been shown that the formula of albumin (according to Schützenberger) is at least  $\text{C}_{240}\text{H}_{387}\text{N}_{65}\text{O}_{75}\text{S}_3$ , corresponding to a molecular weight of 5473.

Egg-albumin dried over calcic chloride contains 45.85 to 46.58 per cent. C, 7.62 to 7.96 per cent. H, 12.14 per cent. N, 1.63 per cent. S, after deducting 2.3 per cent. of ash. It loses 12.76 per cent. of water at 100° to 130°, turning slightly brown even at 100°. At 165° it suffers no further loss, and at a still higher temperature empyreumatic ammoniacal decomposition-products are formed.

The albumins thus far analysed were all mixed with the plasmin, which can be removed by dialysis and filtration, or dilution, neutralisation, and carbonic acid; and with the nitrogenous body which passes with the salts into the dialysate and holds phosphates and free earths in combined solution. The pure soluble albumin of Graham has not yet been subjected to elementary analysis. It is therefore premature to speculate on the formulæ derived from the analysis of the coagulated impure albumin, although we may hope that the information already gathered represents something like the beginning of a truth.

*Other properties of soluble and coagulated albumin. Effects of heat upon coagulated albumin in the presence of water.*—Hard-boiled white of egg heated to 150° with water for two or three hours, dissolves and forms a solution which is precipitated by acids (Wöhler, *Ann. Pharm.* 41, 238). When it is heated to near 200° with water in a copper digester, the walls of the vessel become covered with a film of cupric sulphide, and the albumin dissolves, with exception of a portion which remains unaltered. The solution contains a little free acid, and substances partly soluble, partly insoluble in water. When coagulated albumin is boiled for some time in water it swells up strongly, softens, decomposes, and gives up animal matters to the water (Gmelin).

Mulder (*Ann. Pharm.* 47, 314) boiled white of hens' eggs in a Papin's digester for 150 hours, and obtained a solution as well as an undissolved portion, which latter Mulder declared to be unaltered albumin. It contained 54.34 per cent. C, 7.16 per cent. H, and 15.33 per cent. N. The watery solution on evaporation left a residue, which after exhaustion with alcohol, dissolved in water and gave a precipitate with neutral lead acetate and ammonia. Both precipitates were decomposed by hydrothion, and yielded a substance which Mulder termed protein trioxide and found to contain 50.75 to 51.36 per cent. C, 6.69 per cent. H, and 15.01 per cent. N. It was neutral, soluble in cold water, precipitable by dilute mineral acids, chlorine and metallic salts, but not by acetic acid, potassic ferrocyanide, ammoniac chloride or barytic chloride.

*Effect of electrolysis upon undiluted white of hens' eggs.*—Many experiments have been made upon this subject, but with

the exception of the one of Lassaigne, never upon pure or purified albumins. Arnim (*Gilb.* 8, 259) and Marx (*Schweig.* 54, 209) found albumin alone or diluted with water to be completely coagulated at the negative pole. With a strong current, a rapid deposit takes place at the negative, and a slower deposit at the positive pole; with a very weak current, there is a deposit at the positive pole, while an alkaline solution of albumin is formed at the negative (Brande, *Gilb.* 64, 354). With a weak current C. G. Gmelin (*Gilb.* 64, 347) observed coagulated albumin at the positive pole only, never at the negative; a strong current caused coagulation at both poles, which Gmelin attributes to the heat evolved. Lassaigne (*Ann. Chim. Phys.* 20, 97) precipitated albumin with alcohol, washed the precipitate with weak spirit to free it from chlorides, and submitted its aqueous solution to electrolysis, which did not cause coagulation excepting after addition of sodic chloride and then only at the positive pole. According to Morin (*Par. Soc. Bull.* 1861, 104) this result is due to the low conducting power of the liquid. According to Smee (*Proceed. Roy. Soc.* 12, 399 and 505; 13, 350) fibrin is formed by the electrolysis of egg or serum albumins; and Wittich (*Journ. pract. Chem.* 73, 18) also subjected undiluted white of hens' eggs to electrolysis and obtained a dense white layer of coagulated albumin at the positive pole, and when the experiment was continued long enough the negative pole became covered with a clear jelly of albuminate of soda, which last did not appear with diluted albumin. Solutions of alkaline albuminates likewise deposited the albumin in films at the positive pole; acid solutions of albumin deposited it more slowly at the negative pole, in the form of a diffused cloud, while alkali or acid was set free at the opposite pole. When the solution contained free acid, free alkali, or carbonate or bicarbonate of alkali, the deposit of albumin did not take place, or took place very slowly; but when the solution contained neutral salts, such as sodic chloride, sodic phosphate, sodic sulphate, potassic sulphate or nitrate, the deposit at the positive pole was much more abundant.

*Putrefaction of albumin.*—Natural albumin putrefies with evolution of hydrogen, nitrogen, carbonic acid, and hydrothion, the quicker the higher the natural temperature to which it is



exposed, provided it is protected from being dried up by currents of air and other conditions favouring rapid exsiccation; the presence of added water favours rapid decomposition. Coagulated albumin does not quickly putrefy even under water. The earliest systematic experiments for ascertaining the products of putrefaction of albumin were made by Bopp. Albumin was dissolved in 40 to 50 parts of water and exposed to the air for a few weeks, at the temperature of 25°. The gaseous products were apparently not noticed, the other products were separated as follows.

*a.* The liquid was distilled with milk of lime, and yielded ammonia and a body of penetrating odour, part of which passed over in crystalline scales when the ammoniacal liquid was distilled in its turn with phosphoric acid, while another portion was extracted from the distillate by ether. It was very volatile and assumed a rose-red to brown-red colour with dilute sulphuric acid, forming oily drops.

*b.* The residue containing lime was distilled with a slight excess of sulphuric acid, and yielded volatile acids.

*c.* The liquid resulting from the last operation was mixed with neutral lead acetate, by which there was thrown down, besides the sulphate of lead, a plaster-like precipitate from which hydrothion separated brown acid oily drops.

*d.* When the liquid remaining after the separation of the plaster-like lead salt was freed from lead by hydrothion, it yielded on evaporation a viscid syrup, which was resolved by alcohol into leucin and a soluble portion. The latter was free from sulphur, had an acid reaction, formed with lead oxide a salt soluble in alcohol, and when boiled with sulphuric acid yielded tyrosin and a brown colouring matter.

The subject here under consideration will be further treated of in other essays of this volume. The limitation of our space does not allow us to complete this exhaustive survey. This, however, we hope to do in a future edition of the Annals.

## XIV.

*ON INFECTION, AND THE VARIOUS STATES OF  
AGGREGATION OF INFECTIOUS MATTER.*

THE consideration of infection is a part of medical ætiology, or that branch of medical science which treats of the causes of diseases. It is therefore by no means, as is not rarely supposed, a merely chemical, or as is maintained by others, a microscopical question. For some processes of infection are by no means chemical. Thus a most important lately discovered disease, viz. fleshworm disease or trichiniasis<sup>1</sup> is propagated by the transfer into the human (or animal) intestine of living wormlets, which there propagate, and their young migrating into the body cause the general effects of the disease. Here the infection is due to a living parasitical animal species.

In other diseases no such well-defined beings can be ascertained to exist, and however curious may be the resemblance which such diseases bear to parasitical diseases, the individual agents, by which the infection is carried, maintained, and reproduced have not yet been discovered. Accordingly medical inquirers are in a state of doubt and perplexity. The doctrine accepted by many during the last thirty years according to which the ordinary infectious, febrile diseases were mere chemical processes, and engendered by the introduction into the body of agents which were likened to ferments, then believed to be unorganised, has been somewhat shaken by the development of the views concerning ferments properly so called, their division into two classes, of which one was termed shapeless or non-organised, the other shaped or organised. This development led many ætiologists into the paths which their

<sup>1</sup> For a complete account of this disease, see *Rep. Med. Off. Privy Council*, 1865.

science has repeatedly taken on former occasions, namely, to the belief that disease-causes were *parasitical beings* of a specific kingdom amongst things extant and organised; that all living things had to be divided into three kingdoms instead of as hitherto into two only, namely into animals, plants, and disease-causes; and that the latter being, like animals and plants, things created, or, speaking less dogmatically, of the same origin as animals and plants, had an independent existence as beings, and infected the individuals of the other kingdoms in the same manner as parasites.

In fact pathology became again to some extent, under the impulse of these hypotheses, what it had been repeatedly before, namely *ontology* (doctrine of disease-causes, as separate beings, to be distinguished from the doctrine which considered the disease (groups of symptoms produced by the cause) as a parasitic being). The philosopher who ponders over the great number of different diseases to which organised beings are subject, and who particularly appreciates the specificity of diseases, that is to say their peculiar unchangeable character within certain limits, and their exclusive attachment either to men, or to species of animals, cannot but be forced to consider diseases with the aid of the ontological hypothesis. But on the other hand, when he investigates the reasons which speak against the hypothesis, he perceives that there are many and strong reasons which prevent him from accepting the hypothesis as a generally applicable or valid one. As it is impossible for him to draw general conclusions concerning a whole field of morbid phenomena, so also he cannot devise measures for the prevention or cure of diseases as a mass. He is reduced to the necessity of studying each individual disease by itself, and adapting to every single case what measures are possible; but the measures, equally with his views of the nature of the case, are mere approximations of his mind by means of a reasonable hypothesis.

In times when epidemics of infectious diseases prevail, society takes the alarm, and many persons then endeavour to protect themselves from infection by leaving the places where the disease prevails, and going to places supposed to be free from it. In times of less alarm, all society, and in times of epidemics that part of society which cannot migrate, rely upon

chemical means either to destroy the infecting matter out of the body (disinfectants), or to stop the infection which has already reached and affected the body (specifics, medicines). The use of these agents is mainly the result of chemical hypotheses concerning the nature of the causes and products of disease. As regards the applicability of these agents it is of no account practically whether infections and products of disease be living agents or whether they be chemical matters possessed only of the powers pertaining to chemical ferments having no organisation or specifically shaped individuality, so long as the chemical means adopted for their destruction are strong enough to destroy either.

Infection, as the act or process committed by a thing or specific being, admits of a definition. Such a definition can be correct only within the limits of positive science concerning the subject, and must, if possible, not be liable to any controversy. The strictest logic has to be applied, the use of words has to be restricted to its most legitimate limits, and no latitude or uncertainty of expression can be allowed, which would lead to confusion.

*Infection is the process or act by which an animal or a man receives into his body a substance or matter, or agent, which is capable of producing disease, and is actually thereby induced into a diseased state; the disease being admitted, for the purposes of this definition, to be specific and its causing agent to be self-reproducing.*—It follows from this that the accession of mere dirt, technically foreign bodies, the collection of mere impurity, the contamination of the air by offensive effluvia, the pollution of water earth and air by the offal of human operations or the excretions of man and animals, *do not* constitute infection; and it also follows that the counteracting of these influences, the removal of these pollutions and contaminations, must not be termed disinfection. For disinfection being the negation or counteraction of infection, can only be defined as an operation which destroys matter capable of producing infection as above defined; now as these influences are unable to produce infection, the counteracting or destroying them is not disinfection. Infectious matters are carried by men and animals: they may be contained in and about places and



things. Men, animals, places, and things may receive and harbour infectious matters, change them in a manner chemically and physically, or retain them without change. The infectious matters may from these temporary abodes pass again into animals to produce their specific effects, or may be destroyed. Places and things carrying agents of infection are also termed infected, although they are only carriers of an infection imported, and not like men or animals (passive) reproducers of such infection.

In practice processes which could truly be termed disinfections have not been applied to the interior of infected men or animals, but only to their external surfaces, their fur or hair, or to the clothes or uncovered surfaces of men's bodies such as faces and hands or to places and things. Only few carried the doctrine of disinfection farther into practice, and gave strong disinfectants internally, such as chlorine water in enteric fever. This practice is now a thing of the past. If lately a chemist revived a similar practice, and caused to be injected into the blood-vessels of cows affected with the cattle plague watery solution of carbolic acid, it was a proceeding for which he had no medical or scientific authority, and the death of the cows showed the futility of the practice. In a German university some men were disinfected by having their bodies anointed with carbolic acid. The death of the patients was the result of this rash and deplorable practice.

Examples of this kind (of which more might be quoted), show that substances termed (by erroneous hypothesis) disinfectants, have no specific counteracting effects upon infections; that they do not destroy infections as quinine destroys intermittent fever; but that they are only agents of general efficiency which they exercise upon living, healthy, diseased or dead chemical matter; and that in a direct proportion to their general power over all these matters stands their special effect upon infectious ones. Now whereas some of these agents employed for the destruction of infectious matters have actually opposite properties, *e.g.* one preserving organic matter while withdrawing it from the influence of oxygen, the other supplanting or assisting oxygen in the destruction of these matters which the first one preserves, we learn, in a manner *ex juvantibus et nocentibus*,

what we can also ascertain otherwise, and in fact have already admitted in the foregoing, that the nature of infectious matters, or of infections, contagions, and miasms is with few exceptions unknown.

From this the conclusion may be drawn that it is at least useless to try to affect materially that which does not present itself materially to our senses. But practical necessities cause us to dispense, for the time being and pending inquiry, with data, and to substitute for them hypotheses; with the aid of these we conjecture the possible and the probable, and to the results we adapt certain agents of known power.

*On the various modes of infection.*—It is useful to consider the simplest cases of infection, in the first instance those in which the direct mechanical process is known and undoubted. Hydrophobia, as an example, is transferred mechanically upon man by the dog. It is transferred from dog to dog most commonly by the same method by which it is transferred to man. But whether amongst dogs transfer (by inoculation) by a bite is the only mode of transfer, or whether there are other modes of transfer (as is probable from the experience of certain kennels in which rabies becomes general and causes the necessity of destroying the entire kennels) or whether a dog may become affected with rabies spontaneously is not at present known.

In the special case of a man who receives a wound from a mad dog, a certain amount of saliva enters the wound, in which an infection is transferred, which works out the death of the man. In such a case we are in the habit of cauterising the wound so as to destroy, as we believe, chemically, the poison adhering to it, and protect the rest of the body from its agency. Or we cut out the piece of flesh in the midst of which the wound is situated, if we have some reason to fear that the poison might in part already be absorbed. We concentrate the whole of our activity upon the isolation of the affected saliva, and upon its destruction, but we do not attempt to disinfect the body after the bitten man has become hydrophobic; we have at present no means of destroying the infection of hydrophobia within the body of either man or animal.

While hydrophobia is distinctly carried by a *morbid poison*, a *product of disease*, snake poison, on the other hand, is not a

product of disease, but a normal secretion of organs specially provided and arranged with purpose and effect. We range it in our classification of hurtful substances with poisons proper. Snake poison acts almost immediately ; there is no time of incubation ; no long period elapses between the insertion of the poison and the production of its effects. It acts in proportion to the quantity in which it is inserted in the wound, and when that quantity is exhausted its effect ceases. Not so the hydrophobic poison or infection ; its effect is scarcely ever immediate ; a period however short, but which may be prolonged to months and years, always elapses between the bite and the outbreak of the disease. This is one of the diagnostic features of specific contagions, that they require a time of incubation, a time during which, so to say, the eggs of the disease laid in the body, are being hatched, a time which is mainly used for the multiplication of the contagion, and the termination of which is indicated by the earliest objective symptom of the outbreak of disease, a rise in the temperature of the body from the normal towards the febrile.

We have alluded to a natural poison inserted by teeth in order to contrast it with a diseased contagion inserted by teeth ; we have thus shown that in the production of hydrophobia something passes into the body which is being infected ; a germ is transferred, a something which acts as the beginning and centre of a portentous effect : we must hold fast this lesson in the consideration of other diseases, in which the transfer of germs by some means or other becomes probable, has to be assumed, but cannot be proved ; at least not in the same direct manner as in hydrophobia. While we thus clearly understand that contagion consists in the transfer of a small portion of disease-producing matter from one person to another, we in no case, pig typhoid and splenic apoplexy in the ox and anthrax in man excepted, know the nature of that matter by which the transfer is immediately effected.

The 70,000 cases of successful infection with typhus and typhoid fever poison which occur annually in this country, and an at least equal number of smallpox, scarlet fever, and other similar infectious diseases, are all assumed to be produced by the transfer of a small particle of disease-producing matter from

one individual to another. If we were to assert with the late Dr. Murchison, the author of a treatise on Continued Fevers, that the poison or infective seedling of typhus was 'probably a compound ammonia' we should not be nearer to a true knowledge of the body in question. It is necessary to be quite clear on this point, that of the exact nature of the infecting agents of the most important diseases including typhus and cholera we know nothing. And the clearer this is enunciated, the nearer are we to the possibility of finding something about them. For as they are unquestionably tangible matters, unquestionably chemical compounds, whether organised or hemiorganised (Frémy) consisting of three or four elements, perhaps five or six, and as the elements so contained in their composition preserve through all changes of molecular form the character of chemical elements, we comprehend that these matters admit of the same amount of definition as any other matters of organic or inorganic nature which enter into the researches of biologists or chemists, and that even should these matters belong to an independent range or grade of things standing to the human and animal body in the relation of parasites, even then they would obey laws of organisation of their own, even then their constituent elements would obey the general laws of matter.

*Infection by definite acts and agencies; inoculation.*—We come nearer to the comprehension of the nature of disease by considering the variety of means by which it may be propagated. Knowing that diseases may at times have a more virulent, at other times a more mild character, and knowing on the other hand that persons who had once been affected with a disease of the particular kind would not be liable to be infected by it again as before, some men of particular wisdom and courage (the earliest ones living in remote parts of China and in remote times) have availed themselves of this information, and inflicted upon persons under their control mild infections to escape severer types and obtain immunity. The oldest example of this kind is smallpox. Smallpox is the most infectious of human diseases; it spreads through families, villages, towns and countries with great rapidity, and in this respect only one disease, cattle-plague, is equal or superior to it. Having recognised the refined insidiousness of smallpox and that they



had no escape from it, and that there were mild types and severe types, men argued that as they must have the disease at one time or another, they might as well produce it upon their bodies when it was of a mild type, and thus procure immunity; moreover they could select the season at which experience shows that all diseases are the most tractable or are suffered with the least domestic inconvenience. The earliest mode of inoculating, or rather intentionally imparting, smallpox, consisted in blowing into the nose of the person to be infected some dried and powdered matter from smallpox pustules. Physicians continued to experimentalise and discovered inoculation. For this great result of reflection and experience, for this most courageous resolution, which contains the germ of the practice we now know as vaccination, mankind is indebted to the Chinese. Amongst the Chinese smallpox was inoculated as early as the year 1000 of our era. The Hindoos learned the practice from the Chinese, and some of their tribes used the marks left by the pustules to inscribe upon the foreheads of their children a mark of the particular caste of which they were members. Through the Hindoos the practice came to the Persians, Armenians and Greeks, and at the beginning of the last century the practice of inoculation became common amongst Greek physicians at Constantinople. There it was noticed by Lady Mary Wortley Montague, and through her letters it was made known in England, and Western Europe. It then became a common practice amongst the rich, and an English physician received a fee of ten thousand pounds for inoculating the children of a Russian Empress. After the discovery and introduction of vaccination, however, inoculation was made illegal, and vaccination compulsory, and in the present day 95 per cent. of all those born and living during the first year are protected from smallpox by vaccination.

*Varying states of aggregation of infectious matter.*—In order to obtain an approximation to an accurate idea of the nature of infectious matters, particularly the specific poisons of diseases, it is necessary to consider their various states of aggregation. The matter which carries smallpox must be capable of assuming an extremely minutely divided state; but it also exists in an apparently fluid state, inasmuch as fluid (lymph)

taken from the poeks themselves is capable of transmitting the disease. Such lymph might, however, be supposed to contain the infectious matter in a particuilar state and not in solution. Chauveau's experiments on vaccine speak in favour of this idea, Bert's experiments on splenic fever blood against it. The infectious matter also exists in a dry and solid state in dried lymph. Other specific infectious matters are capable of existing in the same different states as smallpox; for example, cowpox. The lymph from cowpox can be preserved by being dried upon a lancet, or upon a chip of ivory, or soaked in a thread. Thus fixed it will retain its power of exciting vaccine pustules through the medium of a wound in and around that wound for a considerable time, nay it seems as if such poison in a dry state had an increased power of endurance against destructive influences. Dr. Budd of Bristol caused some vaccine lymph dried upon so-called ivory points, to be enclosed in glass tubes, and the latter to be sunk into the centre of a leg of mutton near the bone. The leg of mutton was then roasted in the ordinary fashion in the kitchen. The tubes were afterwards drawn forth and the ivory points used for infection, which proved perfectly successful. It is consequently clear that a higher temperature than the ordinary one does not always destroy the activity of lymph in the dry state. The experiment was no doubt made under such conditions that it is not necessary or possible for us to assume that the lymph had reached the heat of the coagulating point of albumin ( $64^{\circ}$ ). It was almost certainly below the coagulating point of myochrome (about  $90^{\circ}$ ), and then it was under peculiar conditions, which admit no direct conclusion as to what would have been its fate if it had been heated only to the coagulating point of albumin in the moist state. Dry white of egg can also be heated to a much higher point than moist or dissolved white of egg without undergoing any change such as is expressed by a change of its solubility. The heating experiments on dry vaccine are therefore not conclusive as to the effect of heat upon lymph as it comes from the pustule.

Infectious matters are not supposed to exist in a gaseous state. When contained in liquids they may, as already stated, be considered as dissolved or suspended; in the solid state they may be free or combined, retaining their specific energy in the

combination, as quinine does in the sulphate. What has given rise to the idea of infectious matters assuming the gaseous state is probably their extreme divisibility, by which they assume the form of very fine dust, and are easily carried over great distances. The class of infectious matters which present these characters of apparent volatility, fluidity and solidity, and can pass from one to the other, we will term *volatile inoculable infections*. Of such infections smallpox is the type, and there is only one disease of animals at all comparable with it, and that is the cattle-plague or rinderpest.

There are a number of human diseases, which although producing volatile infections, are not proved to be inoculable, such as scarlet fever, whooping cough, (diphtheria?), croup, typhus fever, and typhoid fever. This class of diseases we range under *volatile infections not proved to be inoculable*. Distinct from these we have another group of infections which we will call *the less volatile, more fixed, not proved to be inoculable*; the type of these is yellow fever. If we go on to consider all other diseases from the same point of standing, we are obliged to construct some more classes; we thus get the important class of *fixed infections, contagious and inoculable*. These cannot assume the volatile condition, and hence can be propagated only by bringing into actual contact with the subject to be infected a certain amount of the fluid or suspended infecting matter. Amongst these the representative is syphilis. Then follow the diseases of animals transferable upon man, namely glanders, hydrophobia, pustula maligna, and vaccinia. For however analogous vaccinia may be to human smallpox (so analogous that some believe vaccinia to be only human smallpox modified by transmission through the cow), it differs from it in this important point, that its poison is not transferable from man to man in the volatile state. The insertion into his skin-tissue of a certain amount of vaccinia is required for man to become infected. In those agricultural districts where vaccinia was first observed it was and is now generally believed that the transfer of the vaccinia upon man takes place by direct contact, and the observations of Jenner mostly referred to cases where, in proof of this assertion, the pustules were observed upon the hands. The hands of the milkers were employed upon the

udders of the cows, and thus the disease was probably transferred. Whether vaccinia is volatile, so as to be transferable from cow to cow is not known, but it is very likely. The cow can be inoculated with human smallpox, but human smallpox poison does not appear to affect cows spontaneously.

All the above infections are specific diseases of a type which within certain latitudes and limitations is unalterable. The following disease however is not, perhaps, so specific although more fatal, and may be called *a volatile and fixed infection, contagious in one sense*, and that is the pyæmic infection. It is undoubtedly volatile, inasmuch as it may be contained in overcrowded hospitals and fly from person to person, infecting those who are slightly wounded as well as those, and more particularly those, who are severely so. But it apparently only enters through suppurating wounds, and is as yet not proved to be inoculable; that is to say, we have no proof that by taking a small portion of pus from a pyæmic person and inserting it into a wound made for the purpose of infection on a healthy person the disease can be reproduced. There is however such experience upon animals, although the first material used in such experiments is not generally taken from animals suffering from pyæmia, but consists, in accordance with certain ideas and facts concerning pyæmia, of simply putrescent matters having no claim to be termed products of disease.

The disease-cause of pyæmic infection seems analogous to that of cholera in this, that before taking effect upon an individual it has to be fermented or caused to undergo a change, and that change is effected in a wound of which the person to be infected with the pyæmic poison must be the bearer at the time of the infection.

We come now to consider the last of these infections, viz. *corpse-poison, or dissection-wound poison*. This infection is fixed and inoculable only, and no particular state of the body of the recipient is required for its successful transfer. This infection leads to destruction of parts and death of the individual in many cases, but it lacks the specificity which is peculiar to other diseases.

Notwithstanding much that has been written and said about cholera, it is difficult to give it a place in a system like the one



which we are endeavouring to construct. We are not certain whether it is volatile or not; whether it may be volatile at one time and fixed at another; for if it be volatile at all it is only moderately so, and in that respect resembles yellow fever. The experiments of Thiersch led to the conclusion that the cholera poison originated in the excretions of cholera patients. They had no power of infection in their fresh state, but only gained that power after exposure to the atmosphere during a number of days. Two days was the shortest time required for cholera excretions to acquire the power of producing morbid effects upon such small animals as dormice. Intestinal contents from the dead body required sometimes seven days before they exhibited morbid action in dormice; after sixteen or seventeen days from the beginning of the exposure to the air, the evacuations ceased to exhibit any poisonous effects. It is by this acquired and passing poisonous nature of the excretions, that cholera resembles pyæmic infection, as above mentioned. These experiments of Thiersch upon dormice were repeated in the Pathological Institute, and proved to yield the same results as those obtained by this observer. These experiments we shall have to consider when we come to treat of some chemical features of cholera. At first sight they lead to the thought, that cholera, being capable of affecting dormice, is not a specifically human disease, but participates more of the nature of poisoning. But this is negatived by other data to be discussed. Cholera in dormice is similar to the disease as it appears in man, but it is not identical with it in all its symptoms; the symptoms are modified.

The Oriental pestilence or plague is *a contagious but not inoculable infection*. This has been amply proved by the researches of Clot Bey. The opposite experience of physicians who inoculated themselves with the pestilence, and actually became infected, is not eogent. Deggio, a Russian doctor in Bucharest, successfully inoculated himself with the Oriental plague, and recovered, while Dr. Whyte, an English physician in Egypt, died from the plague, the result, so it was believed, of his own undoubted inoculation. These cases may be either exceptions, or cases in which an infection took place by the side of inoculation; they are contradicted by a great number of experiments

in which inoculation of plague poison into healthy bodies was followed by no results.

*Views of the chemical school of Liebig.*—During the last thirty years infection has been considered as a species of fermentation; in consequence diseases of this class were termed zymotic. Fermentations were considered to be caused by what was termed *contact action*: a body in a state of change (ferment) was said to communicate its state of motion to another body (fermentee) and cause its decomposition. These ferment actions, and so-called catalyses in inorganic nature, were all used to explain infection. When the fermentations were again ascribed to the life-action of living organised ferments (yeast), then the different contagions were also assumed to be referable to organised ferments. Many new ferments were supposed to be discovered, such as produce putrefaction, souring of milk, lactic acid, decomposition of urine, of wines and many other substances. This went so far that the fermentative effects in the living body, such as pancreatic action, were ascribed to the presence of living ferments in the gland. Gradually the non-shaped ferments, such as diastase, emulsin, pepsin, pancreatin, were separated from the supposed shaped and organised ones. Some non-shaped ferments, previously believed to be organised, had to be admitted to be products of the organised beings which did not themselves participate in producing fermentation. (Musculus). All these contentions will be more closely considered in other articles.

## XV.

CONFLICTING VIEWS OF CONTAGIONISTS AND ANTI-CONTAGIONISTS CONCERNING THE ORIGIN OF INFECTION. DIPHTHERIA IN ANIMALS. (*Summary and Additions.*)

THIS subject has been well discussed by Dr. Thorne in a paper read before the Epidemiological Society, April 8, 1878. He put the question whether any case of acute specific fever could arise independently of an antecedent case. By acute specific fever he meant any febrile disease capable of reproducing its own kind; the power of the disease of reproducing itself is therefore admitted, the question is its origin *de novo*.

Those who maintain that no case of infectious disease can arise independently of a prior case of the same disease (and these are sometimes termed contagionists), support their contentions by arguments of the following kind. They say that infectious diseases do largely owe their spread to communication, immediate or mediate, with prior cases of the same disease. With regard to many of these diseases we know, says Dr. Thorne for the supposed contagionists, what are at least the ordinary means by which their producing causes are eliminated from the bodies of those affected by them, and these producing causes we can trace, at times through numerous and intricate channels, until at last we find that in some way or other they are received into the systems of some hitherto healthy persons, in whom forthwith the diseases they give rise to are reproduced with all their well-known signs and symptoms. It may then be urged that this *ordinary* source of infection is the *invariable* one, and may be maintained to have been open even in those cases where investigation has led to no result.

The contagionists further point to the fact that new channels

by which the producing causes of these specific diseases can be conveyed from one person to another are periodically being discovered; and that consequently outbreaks, which formerly would have been brought forward as affording indubitable evidence as to spontaneous origin are now known to have been due to the conveyance of the poison by means of some hitherto unknown channel. Thus since it was ascertained that enteric fever and scarlet fever could be transmitted through the agency of milk as a vehicle, numerous outbreaks of these diseases, particularly of enteric fever, have been ascertained to have owed their spread to this means, and thus that which must formerly have remained unexplained on the hypothesis of the contagionist, now tends to confirm his views. The same remark applies, according to Dr. Thorne, to the outbreaks of enteric fever, which have owed their spread to that form of water-pollution which in intermittent water-services leads to the specific poison of the disease being drawn with other filth into the water-mains during periods of intermission, and to its being subsequently distributed to the general body of consumers. (To such agency the latest (1878) outbreaks of enteric fever at Croydon are attributed). Therefore, say the contagionists, where all known methods for the conveyance of these producing causes fail to explain the existence of the diseases produced by them, it is fair to assume that the infection has really been communicated by some hitherto unknown channel.

Thus a woman became ill in the presence of a mass of putrid manure carried on a railway truck, and attributed her illness to these emanations; it was however subsequently found that before she had been in the presence of the manure, and well within the incubation period of enteric fever, she had stayed at a house where a fatal case of enteric fever had occurred, and had there been exposed to the very conditions which had led to the outbreak in the house she visited.

Dr. Thorne further urges on behalf of the contagionists, that periods of incubation, ordinarily known to be subject to certain variations in length, do in exceptional instances vary much more than is generally recognised, and in consequence, constitute a source of error for which allowance must be made. Dr. Budd in his work on typhoid fever, refers to cases in which the incu-



bation was probably limited to four and two days, and says that the short period of incubation afforded evidence of the high degree of concentration of the poison received. On the other hand Dr. Thorne relates the case of a female, in which there must have been a period of incubation of enteric fever all but a month in length. In the middle of that period the patient had however for two days suffered from a sharp attack of diarrhoea.

Dr. Thorne also believes that the specific producing cause can remain in existence out of the body, and retain its vitality for a long period of time. The two alleged states of splenic fever are quoted, as described by Pasteur, and it is maintained that the spores of splenic fever, though reduced to dust, melted and dried repeatedly, and kept in putrefying liquids, yet retained their virulence undiminished at the end of four years of such treatment. With regard to scarlet fever Dr. Thorne says there are several instances on record which tend to show that its producing cause will remain in existence out of the body for more than a year, and then act with unabated virulence. Instances have come under his notice in which the facts warranted the conclusion that the producing cause of diphtheria had been retained for months about premises in which cases of this disease had previously occurred. Dr. A. Carpenter, in a paper read before the British Medical Association in 1875, related an experience regarding smallpox, which a practitioner in Oban had related to him as having occurred in Mull. A proprietor of that island ordered the removal of some old houses which had lain in a state of ruin and uninhabited for many years. Eight men were employed in digging the walls, and everyone was attacked with smallpox; the germs of that disease having (been supposed to have) lain in these houses all those years.

The following are further cases illustrating the extremely long vitality of the typhoid fever-producing cause. Dr. W. Ogle, in some of his reports on the sanitary condition of the East Herts district, refers to cases in which attacks of enteric fever have in successive autumns occurred in isolated dwellings, where so far as could be ascertained by the strictest investigation, no new source of infection could have come into operation. Dr. Budd once knew a labourer's cottage which remained vacant nearly two years, in consequence of nearly every inmate of it

having contracted typhoid fever. At the end of that time it was retenanted, and three weeks had scarcely elapsed before several of the new inmates were simultaneously seized with the same fever. The cottage stood alone in a secluded spot, and there was no fever in the neighbourhood at the time of the second attack. Dr. Thorne states that he knows a detached house which stands in a country district, and which was occupied by a groom and his family, amongst whom enteric fever prevailed in the autumn of 1872, one case being fatal. This family continued to occupy the house for nearly two years after this occurrence, but they left it some time in 1874, in consequence of the departure of the owner of the estate on which it stood. From that date the house remained unoccupied until February 1876, when it was tenanted by new inmates; and exactly within fourteen days of these latter taking up their abode there, enteric fever broke out amongst them, and a most careful inquiry led both the medical man in attendance and Dr. Thorne to the conclusion that the disease was not imported. Dr. Thorne also knows an isolated parsonage in which the families of two successive vicars have been attacked with enteric fever. When I (the Editor) studied the causes of enteric fever in the town of Giessen, I recorded a history of every house concerning which I could obtain data. In several houses successions of tenants were seized with the disease. The most remarkable house was the official residence of the bursar of the university (*Rentamtman*); in this house three successive families of three successive bursars, were, shortly after having moved into the house, I believe all of them from healthy country towns, attacked with enteric fever; of each family several members died, others were much invalided, and their happiness destroyed for life. If the infection can be supposed to have resided in this house, it must have lain dormant twice during more than twenty years.

The cases of the first two families I learnt by personal inquiry, the last being notorious at the time of my inquiry. The case of the first family I learnt from a son of that family now deceased, when I related the cases of the second and third families to a colleague in the presence of that gentleman; the latter had been down with enteric fever with other members of the family in the very *Rentamt* I was speaking of.

Dr. Gilbert Child has published some evidence regarding the transfer of the infection of enteric fever by means of dirty bedding and clothes. On November 20, 1873, a man arrived at a house in an Oxfordshire village, where his mother and his brother lived, bringing with him several boxes of some very filthy bedding, clothes, etc. On the 29th of the same month the brother was seized with a fatal attack of enteric fever, and on the following day the mother fell sick of the same disease. No source of infection being known, the man referred to was questioned as to his prior movements, and it transpired that in the previous September his wife had died of enteric fever at Toronto, and that he, on subsequently leaving America, had brought with him her dirty bedding and clothing. The only other case of enteric fever which could be heard of anywhere within the district was that of a woman who was attacked on December 5, and she, being also one of the man's relations, had taken to her home some of the dirty things to wash.

That contagious diseases are self-propagating may also be urged in view of the long-continued and complete immunity from them which certain isolated countries have enjoyed, and of the virulence which such diseases have exhibited when once introduced into them. America is believed to have been free from smallpox prior to its discovery by Columbus in 1492; Iceland and Greenland are also said to have been free from it for long periods; the Faroe and Fiji Islands enjoyed a long immunity from measles; but when the disease was imported it spread through them with terrible fatality.

The case of the anti-contagionists is stated by Dr. Thorne with some diffidence; according to him they freely admit that the diseases in question are self-propagating, but they unhesitatingly assert that some of them at least do at times arise independently of antecedent cases, that they are in fact of spontaneous origin. Their opinions derive support from the well-known fact that spontaneous generation of the traumatic infections does apparently take place (*Sixth Rep. of Med. Off. Privy Council*, 1863), and that their respective contagia do arise, settle, grow and thrive in putrefactive wound-products.

Thus if the exudation of a simple (deceptive adjective!) peritonitis be injected fresh into the peritoneum of another animal,

the disease assumes a more intense form in the second than in the first, and if in this way the disease be communicated to several animals at the same time, and the most severe cases be picked out for the further transmission of their exudation, a still more intense inflammation will result, until at last a virus is obtained of which the virulence resembles that of the specific cases of pernicious peritonitis in the human subject. A simple inflammatory fluid is by artificial selection transformed into an apparently specific poison.

The outbreaks of typhus amongst troops when they are ill-fed and badly cared for, are also adduced by the anti-contagionists as supporting their view of the possible spontaneous development of the typhus poison under favourable conditions. But we think this illustration rather unhappy, as with all armies the opportunities of importation of the infection from extraneous foci literally abound.

Next the cases of sporadic disease are adduced, in which the most careful inquiry fails to elicit information justifying the assertion that the disease is due to an antecedent case. There are a number of instances in which trained observers carrying on their observations in isolated localities, free from the ordinary sources of error attaching to life in large towns, and making full allowance for all known sources of error, entirely fail to connect outbreaks of some of those diseases, especially of diphtheria and enteric fever, with any antecedent case, whether near or remote; and hence these observers feel themselves compelled to fall back upon the view that such diseases do, at times, have an independent origin. (See *Reports*, by Dr. Charles Kelly, on the condition of the Combined Sanitary District of West Sussex). Dr. Thorne's failures to elicit any history of previous infection as regards cases of infectious inflammation of the throat—of diphtheria, which have occurred in isolated houses lying in districts far removed from other human habitations, have forcibly struck him, the more so as in the majority of instances those first attacked have been children of such tender years that all their movements could be ascertained with remarkable accuracy.

Instances in which a disease such as enteric fever recurs in successive years, or after a lapse of years, in houses where it has



previously existed,—instances which are assumed by the contagionists to afford evidence of the disease-cause being retained as it were in a dormant condition,—such instances may be explained by the anti-contagionists as due to the circumstance that the conditions favourable to the development of the poisons being present, each separate outbreak is but the result of a recurrence of the process by which a new poison is each time brought into existence. Of course this view excludes the hypothesis of *contagium vivum*, or if not, it includes the hypothesis of spontaneous generation; in either case it has a most difficult position to maintain. Dr. Thorne adduces yet a more powerful argument on behalf of the anti-contagionists to the following effect. He says that those who believe that the poisons of the acute specific fevers may and do arise spontaneously have a perfect right to remind their opponents that there must have been first cases of each of the diseases under consideration, and that unless we are prepared to accept the view that the several contagia, which breed truly by a process said to be as regular as that by which dog breeds dog and cat breeds cat, were originally the result of definite acts of creation, it must be assumed that they in some other way did arise independently of antecedent cases; and what right, they may ask, have we for asserting that a process once possible is now impossible? Dr. Budd says an argument such as this constitutes the last refuge of the partisans of spontaneous origin; but if it be so, says Dr. Thorne, it is a stronghold in itself, and one which must at least be noticed before it is passed by.

Dr. Thorne himself believes that there are some grounds for doubting whether certain of these diseases, as at present met with, are exclusively self-propagating, and for raising the question, whether they may not occasionally arise independently of antecedent cases. Such personal experience as has inclined him towards this view is mainly limited to investigations into the origin of outbreaks of *diphtheria*, and one point with which he has been specially struck is the following. In isolated districts, and in houses situated at times miles away from other habitations, and in some instances lying in lonely spots among mountain-ranges, where a visit to or a visit from the nearest town or village would be a circumstance too important to be

forgotten, Dr. Thorne has met with instances of what appeared to him to be nothing more than a simple inflammation of the throat, at times so trivial that it has passed all but unnoticed, and yet it has led by transmission through other persons to cases of well-marked and severe diphtheria. The first attacks have often happened in children whose former movements could apparently be traced with the strictest accuracy; they have occurred under circumstances which did not appear to admit of previous infection, and it has been difficult to interpret their occurrence except on the supposition that in some way they have arisen independently of prior cases. With regard also to the well-marked attacks of diphtheria to which they seemed to give rise, all other sources of infection could be excluded with a degree of certainty rarely to be met with. And as to those first affected, whose cases appeared to be earliest in a series which led up to attacks of well-marked diphtheria, it has more than once happened that they were children in whom so-called 'sore throat' was a common affection, and whose fauces when examined exhibited a loss of tissue indicative of former throat-attacks.

Dr. Thorne has further noticed that over an area of some miles around the district in which genuine diphtheria was prevailing, there existed prior to as well as during the diphtheria epidemic numerous instances of sore throat which, so far as examination of the patients was concerned, in every way resembled the early cases above referred to, and yet which gave no indication of being infectious; sore throat being in fact in the surrounding district a prevailing ailment. And from this he was inclined to draw the conclusion that conditions very similar to those under which genuine diphtheria was epidemic in a limited district obtained, and had obtained before genuine diphtheria was anywhere seen, over a wide area around the immediately infected locality, and that these conditions, leading to a somewhat general predisposition to simple and apparently non-infectious inflammatory sore throat, had further, probably under somewhat modified circumstances, tended at certain points to produce an affection capable of putting on the property of infectiveness, which thus led to the transmission of the disease in a distinctly communicable form to others.

From such data and explanation Dr. Thorne constructs the hypothesis of the progressive development of the property of infectiveness. And if the contagia of the acute specific diseases belong to the vegetable world, he is inclined to admit that organisms capable of producing a minor and an incommunicable disease in particular stages of their growth, become capable, in the course of their subsequent development, of producing a major disease communicable from person to person; the affair being essentially one of soil.

Dr. Thorne does not consider this to be a question of the development of a living organism out of matter independently of antecedent life, but merely the production by means of a process of evolution of that which gives to an already existing organism that property by which it becomes infective, a property which it may perhaps lose directly it is deprived of the circumstances which favoured its development, in much the same way as special characteristics may be artificially developed in higher plant life, and be as easily lost again.

This hypothesis thus far chimes in with that of Pasteur, according to which the organised cause of splenic fever has two different forms of existence, one in which it is actively infectious, another in which it is quite innocuous; one can be transformed into the other at will. But these data are not conclusive on account of the data adduced by Bert, which will be considered in the next article.

In all these discussions it has been entirely omitted to inquire, whether some of the lower animals could not be permanent or temporary carriers of some of the infections thus far almost exclusively observed in man. I think it therefore of particular importance that I should be able to direct the attention of the reader to some observations on diphtheria in fowls by Nicati of Marseilles (*Compt. rend.* 88, 1879, 297), and to add thereto some notes of observations of the same kind, which I had an opportunity of making in 1875.

The attention of Nicati was directed to the subject by M. Gavard, a veterinary surgeon of Marseilles. A disease had during the winter of 1877-8, decimated a fowl-yard at Longchamp. On examination of the victims it was found that thick false membranes of yellow colour covered either the

mouth and pharynx or the eyes. In one case they penetrated into the bronchi, and the lungs showed yellow hepatisation. A hen died twenty-four hours after the manifestation of the first symptoms, having yet laid the day before. Another of the affected animals lived three days, a third five; some animals were ill during weeks; some recovered.

Nicati extracted some of the materials from the lower layer of the false membranes nearest to the normal tissue, and inoculated it upon the cornea of rabbits and fowls. He made a great number of pricks into each cornea, and then rubbed into them, by means of the lids, the *débris* of the false membranes. All inoculations succeeded completely.

Gibert then showed that during the prevalence of this epizootic disease in the Boulevard de Longchamp, there had been an increased mortality from diphtheria amongst the human inhabitants also. In consequence of this, further inquiries regarding domestic birds were instituted, and the presence of *diphtheria in pigeons* was discovered. In a pigeon-house attached to a villa named Talabot, in the suburbs of Marseilles, many birds died. Two were examined after death; in both the mucous membrane of the back of the mouth was covered with a pultaceous layer; one only exhibited a distinct diphtheritic membrane, which could be easily detached.

Another epizootic, attacking both pigeons and fowls, was observed by Dr. Queirel in a fowl-yard in the Rue de l'Académie in the centre of Marseilles. Of the surviving animals three, a fowl and two pigeons, were examined by Nicati. The fowl suffered from a second attack after having apparently recovered from a previous one a few weeks before. The three animals died from increasing weakness, the fowl after having refused food for twenty-four hours. In all three animals the throat was covered with a false membrane which reached up into the nasal fossæ. These epizootics also occurred during November and December, there being at the same time an increase of mortality amongst the population from croup, membranous angina, and diphtheria. While these epizootics were under his observation, Nicati observed four cases of diphtheria of the eye in human beings. These facts together with the inoculability upon rabbits brought him to surmise that there might be some relation



between the diphtheria of fowls and that of man, and that perhaps they might be one and the same disease.

In the summer of 1875 a friend and neighbour of mine lost many of the young pigeons which were in course of being reared in his pigeon-house. The birds' could take little or no food from the parents, and became emaciated and died. On examination their throats were found occupied, in situations equivalent to the tonsils in man, by thick false membranes of yellow colour and great consistency. The membranes had projections which reached deep into the tissues of the throat, with which they became intermingled; they could be removed with the aid of suitable instruments and cautious manipulation of the pigeon's throat. In the interior the masses showed no particular cohesion, and appeared like masses of favus. The corroded holes from which they were removed looked raw, but did not bleed. After the removal of the false growth the young pigeons again took food, but few were saved by this in itself difficult operation. The parents, which fed the young pigeons thus affected, did not appear to take the disease.

The same disease I observed upon a fowl in my own fowl-yard; the membrane and prolongations into the tissue of the throat, formed a mass of the size of a haricot bean, and pressed the glottis on one side, so that there was stridulous voice and difficulty of respiration. I had the animal killed and examined. The microscopic appearance was the same as in the pigeon: minute particles, in which with a high power of the microscope no organisation was distinguishable, mixed with *débris* of destroyed tissue.

I was credibly informed that a great breeder of fancy-pigeons at Acton had during the summer of 1875 lost a great number of young, mostly half-grown pigeons from the disease described in the foregoing paragraphs.

The question raised by these observations upon animals must be definitely answered before the position assumed by the anti-contagionists can be considered tenable. The observations furnish a new means for studying the nature of an infectious disease experimentally, and for approaching the chemical problem of its zymosis on ground which can be extended at will.

## XVI.

RECENT DATA AND ARGUMENTS IN FAVOUR OF  
AND OPPOSITION TO THE HYPOTHESIS OF THE  
LIVING CONTAGIUM. (Summary.)

AMONGST the morbid anatomists the hypothesis of the living contagium has hitherto found few adherents. There are however a few, and Professor Klebs of Prague (*Amtl. Ber. 50 Versamml. Deutsch. Naturf. und Aerzte. München, 1877, p. 41*) *par exemple* is not only an adherent, but an investigator and expounder of this hypothesis. He believes that the various data already obtained necessitate the assumption that the causes of many and important diseases are to be searched for and found outside of the human body, and that they are, while producing diseases in the body, of a parasitical nature. The parasites are assumed to be 'low organisms' regarding which it is stated more by implication than by direct enunciation that they are plants, of the same category as those which produce processes of fermentation properly so-called. The names of Schwann, Helmholtz and Pasteur are quoted as the scientific expounders of the hypothesis that fermentations are caused by organisms. This hypothesis is considered by Klebs to be something like a proved law of nature, and from it he argues with the aid of analogy in support of the zymotic hypothesis of disease. He first speaks of the close genetic connection which according to him exists between certain forms of gangrene and so-called inflammatory and febrile infective diseases, and argues that this connection made it probable that both diseases had the same mode of origination. The hospital gangrene, and the purulent affections following gunshot wounds, as observed in the last great continental wars, are said to prove this already to the naked eye of the observer. But the final proof is afforded by the discovery

that in the bodies of persons suffering from pyæmia or from hospital gangrene the same varieties of fungi are met with, when the parts are examined under the microscope.

The immediate effects of the presence of these parasitic organisms are either mechanical or chemical. (Here a distinction of the infective process from fermentation is admitted, namely the mechanical action of the supposed fungi determined by locality, which does not take place in ordinary fermentations.) The fungi can produce death by the destruction of important parts, particularly the central nervous system (brain and spinal cord), or the heart. Before the fungi produce death by their effects, these effects cause a series of phenomena on the part of the body which they have invaded. These are termed 'reaction,' and this reaction may in some cases lead to a spontaneous cure. Reaction is not necessary to ensure the healing of wounds, and to have shown this, says Klebs, is the merit of Lister.

It is admitted that a division of the phenomena of a disease into those which are due to the infecting organisms, and those which are reactions of the infected body cannot be carried out with the same precision in all infective diseases; but it is maintained that the proof, that all infective diseases have similar causes can be furnished by three different modes of investigation: 1. By the anatomical investigation of the diseased organs; 2. By isolation and propagation by intentional rearing (also termed 'cultivation') of the disease-germs; and 3. By the reproduction in healthy animals of the disease-process through the instrumentality of such intentionally reared fungi or germs.

The proof of these propositions begins with the consideration of splenic fever in the ox. The Austrians claim that Pollender, and the Russians that Brauell discovered, what the French as we think with the greatest justice claim for their Rayer, namely to have made the first observation to the effect, that the blood of animals which have died of splenic fever contains a great number of little rods, which some claimed to be organisms, others alleged to have the properties of crystals. Rayer, and later Davaine, experimented upon this question, and found that the transfer of a very small number of these rods into the veins of a healthy animal was sufficient to cause the splenic fever and rapid death of the inoculated animal. From this it was concluded that

these rods were organisms endowed with the faculty of reproduction, and that the reproduced generations represented the true cause of the disease. The usual counterproofs were instituted, such as excision of the inoculated part at a time intermediate between the inoculation and the period at which general symptoms would have appeared, if the part had not been excised; the excision prevented the appearance of general symptoms, and of any disease whatever, although it was shown that in the excised part a multiplication of rods had already taken place. Davaine termed the rods bacteridia, and claimed for them to be specific low organisms, and the true cause of splenic fever.

There remained the objection that the inoculated matter contained not only the bacteridia, but also larger or smaller quantities of liquid adhering to them and coming from the juices of the animal which furnished the bacteridia. Such liquid might of course be supposed to be able to act as an unorganised or shapeless ferment. To remove these objections Klebs and Tiegel endeavoured to filter the liquid from the bacteridia, by pressing it through septa of porous burnt clay. They were successful, under what they term favourable conditions, in obtaining a liquid free from bacteridia, which on inoculation proved perfectly inactive. Pasteur afterwards, so it is said, applied the same principle and obtained the same results as Klebs and Tiegel.

The reservation covered by the term 'favourable conditions' just mentioned implies perhaps mainly that in some conditions, which are unfavourable, bacteridia pass through the porous clay-septa; of course such a liquid is unsuited for the experiment here in question. But it does not exclude that other variety of unfavourable conditions, under which a liquid free from bacteridia causes infection. I do not know whether Klebs and Tiegel have observed this amongst their unfavourable experience, but it is certain, and we shall see that other observers have. Indeed Pasteur has thought these contrary experiences so telling that he abandoned the argument derived from the exclusion of the organisms by filtration from the fluid to be tested, and substituted the method of investigation mentioned previously under 2, namely the isolation and propagation by



intentional rearing out of the body of the disease-germs. This might be termed the method of neutralising any possibly adhering unformed ferment by almost infinite dilution; or, this method of freeing bacteridia from the suspicion of carrying splenic fever ferment only as an adhering impurity on their surface by subjecting a series of twelve or more successive generations to pure liquids twelve times renewed may be termed a process of washing, which could not be supposed to leave anything unclean in the sense here required amongst their surroundings. Pasteur found that the epigones of these experimentally reared bacteridia were as destructive as those taken from the blood of the animal which furnished the first stock of organisms.

However, now came this experiment of P. Bert (*Compt. rend.* 84, 1877, 1130) which he performed in the course of his trials regarding the influence of oxygen under high pressure upon ferments, poisons and infectious matters. Splenic fever blood, which did not lose its virulence by oxygen under high pressure, but killed guinea-pigs as before, was mixed with absolute alcohol drop by drop, until to one volume three volumes had been added; the coagulum was filtered, washed well with alcohol and dried *in vacuo*. A piece of this cake killed a guinea-pig, and blood from this guinea-pig killed several successions of guinea-pigs and a dog; the blood of these animals contained no bacteridia since the one poisoned with the alcohol precipitate. The precipitate by alcohol yielded to water the virulent principle, and from this it could again be precipitated by alcohol in the shape of light white flakes. It lost in intensity by this treatment, so as to be unable to kill a dog, but it killed yet three successions of experimental guinea-pigs. From this the conclusion was unavoidable that there is contained in the blood of splenic fever a toxic and virulent matter, which withstands the action of compressed oxygen and of alcohol, and of drying *in vacuo*, and can be isolated in the same manner as the shapeless ferments called diastases.

The fact that oxygen under a pressure of fifty atmospheres does not destroy the virulence of splenic fever blood is a negative which by itself would have no significance. But it acquires a meaning by ranging splenic fever poison with other ferments

which also show this resistance. For according to Bert, fermentations, which are supposed to be due to the influence (whatever that may exactly mean) of living beings (putrefaction, acetification of wine, alcoholic fermentation) are arrested by the influence of oxygen under the pressure of fifty atmospheres, whereas fermentations due to (undoubtedly) dissolved matters (such as diastas, pancreatin, mycosin, emulsin), withstand the influence of this agent. If therefore bodies acting in the manner of ferments, but the nature of which is not exactly known, are tested by oxygen under fifty atmospheres, they will exhibit either liability to be changed, i.e. destroyed, or remain unchanged. If they remain unchanged, they show analogy to the shapeless non-organised ferments; if they are destroyed, they show analogy to the ferments at present most frequently admitted to be organised. Now splenic fever blood sides with the shapeless ferments by remaining unaltered; the same is the case with the poison of the scorpion, and the infection-matters of vaccine and glanders; by this test these infection poisons appear *primâ facie* as non-organised.

These experiments of Bert created the utmost consternation amongst the adherents of the hypothesis of the living contagium. For splenic fever had been the strongest support of that hypothesis, no other demonstration approaching that which its materials furnished in certainty of effect and distinctness of form. Moreover it fulfilled a condition upon which alone the hypothesis of an acute vegetable parasitism, developing itself by multiplication of the parasites within the body, would be admitted by botanists well acquainted with the low forms of vegetables; the condition namely that the disease should either end shortly in death or be incapable of spontaneous cessation, that is, be in the individual once seized for the rest of its lifetime, while quite able to destroy life by a chronic course such as obtains in glanders. These conditions of death or incurability were admitted about 1872 by De Bary to be good criteria for vegetable parasitisms.

Upon the evidence before us thus far we should be able to explain splenic fever as a zymotic infection, for the perpetration of which, so to say, the supposed shaped ferment of the parasitic bacteridia is not essential. And if this were true, there

would be an end at once of the better half of the hypothesis. For the other diseases in which parasitic organisms are stated to have been discovered, offer so many difficulties in their nature and course to the identification of their essential causes with these organisms, that it is at present impossible even to construct a plausible hypothesis embodying all the known data; we are therefore far from a theory of acute febrile diseases of man.

The filtration of an infectious poison through porous burnt clay, even if it keep bacteria and other organisms on the side where the liquid enters the septum, does not necessarily permit the liquid to pass unaltered, and does not necessarily allow any and every liquid to pass, merely because there is some pressure behind it; no amount of pressure which does not burst a membrane (bladder) will drive alcohol through it; and it is quite conceivable that shapeless ferments should have as little affinity for clay-septa, as alcohol has for membranous septa, and colloids have for parchment paper, or sized paper. Indeed it is difficult to see by what manner of collateral argumentation the filtration through clay-cells of infectious liquids can ever be raised to the value of a positive demonstration; and unless it be so improved it has only a small force in the shape of pointing to a probability; the objectors may always say, that the porous septum retained both the shaped and shapeless ferment (observe, a ferment isolated otherwise by chemical proceedings, and therefore present by the side of the organisms supposed to be also ferments by the other hypothesis) on the near or entrance side of the porous clay-septum.

Klebs and Tiegel claim to have diagnosed and isolated the microscopic organism which causes the septic condition after wounds; they term it the *microsporon septicum*, and remove it out of infectious liquids by the filtration through porous clay already alluded to, and by other means, which are confronted by many opposing considerations. Pyæmia is infectious, no doubt, but the least infectious of the diseases here most to be considered; it is also the least typical; but it fulfils a condition already stated above, namely that it is nearly always fatal.

Klebs and Soyka further claim to have applied the same process of exclusion to a series of organisms which according to them are originators of inflammation, such as the various

forms of pneumonia, nephritis, carditis, and the acute forms of rheumatism or rheumatic fever. To these organisms Klebs has given the genus name of 'monadines,' the species being perhaps left to be signalised by adjectives derived from the diseases to which they are supposed to be specific.

Other diseases there are in which other forms of organisms supposed to be specific have been found; one of them is relapsing fever, in which a spirally twisted formation and from its shape termed 'spirilla' is observed in the blood (Obermeyer). But here the connection of the disease with the spirilla as cause is more problematical than in any other disease where an undoubted peculiar anomalous formation supposed to be an organism has been found. For the spirilla is quiescent or entirely absent at intervals in the disease, and only appears at periods of exacerbation, or as the expression goes, it wells up during the relapses.

The virus of the cowpox, so accessible to microscopic studies, has never yet been pronounced to be an organised body or organism, i.e. body consisting of organs. The argument in this case has hitherto been confined to inferences.

Chauveau experimented upon the lymph taken from cowpox, and came to the result that it consisted of solid particles, and of a liquid, a result which had always been admitted by microscopic observers. He then allowed the lymph to repose in high cylindric vessels, and testing it by performing inoculation with samples taken from various strata at various times, he ascertained that the infecting quality of the lymph decreases as the solid particles become deposited; that the upper layers of the lymph can lose their infecting qualities entirely, while the infecting qualities of the lower layers containing the sediment increase at the same time. This mode of separating the infectious particles is equivalent to filtration; its result excludes the presence of a dissolved disease-ferment.

Klebs terms diphtheria an accidental disease incidental to wounds, as well as a primary affection (i.e. occurring without wounds); he deplors the losses which the medical profession annually suffers through this disease, particularly amongst surgeons, and says that in some countries diphtheria has seriously thinned the ranks of the younger generation even to the extent



of threatening the extinction of families. According to him there is in this disease constantly present a well-characterised form of fungus, the specific action of which has been proved by specimens cultivated out of the human body. But what is said in the foregoing lines is almost all that we are vouchsafed to know; the fungus goes by the name of the *microsporion diphtheriticum*; it receives the attention of some disinfecting experiments which seem to show (a) that after it has been for a few hours in contact with a solution of sodic benzoate it loses its energy; (b) that animals which have been impregnated with sodic benzoate (it is not said how) to the extent of one per mille of their body weight are not a suitable soil for the vegetation of these fungi. We may here remind our readers that the benzoate was tried in the cases which occurred in the Grand-ducal family at Darmstadt, and found to be without any influence on the disease.

Of lepra Klebs speaks with much preamble and little essence; there are fungi active in this disease, but they are least so where the cellular growth is most developed. Bidentkap of Christiania, Sweden, excised a nodule from a leper, and in this Klebs was able, without much difficulty, to ascertain the presence of groups of bacteria, which in their forms and arrangement differed from the bacteria found in other diseases. To syphilis also Klebs attributes a 'bacteric origin,' which he has lately investigated at length by inoculating experiments upon monkeys. In a postscript to his essay Klebs says that he felt it a command (of the intellect, we suppose) to formulate the parasitary theory of the infectious diseases according to the available observed facts, but that he had not claimed unconditional validity for it. Indeed he now comes to term what he before called a theory, an hypothesis, and demands liberty to change his opinions when new facts might make such a change desirable. Such are the opinions taken of the parasitological hypothesis by a professor of morbid anatomy brought up in the views of the Berlin school, and advanced to the point which he has reached by his personal experience amongst large opportunities.

## XVII.

ON THE SPECIFIC ALVINE FLUX OF CHOLERA, WITH  
SPECIAL REFERENCE TO ITS INORGANIC CONSTITUENTS.  
WITH HINTS CONCERNING THE THEORY  
OF CHOLERA. (*From the Pathological Institute.*)

MANY pathologists admit that the most characteristic sign of cholera is the evacuation of the peculiar liquid discharges, which owing to their similarity as regards external appearance to a decoction of rice, have been termed rice-water discharges. It might therefore be expected that this rice-water discharge of cholera contained the characteristic products of the chemical process in which cholera consists or by which it is accompanied. This process is, in the beginning of the disease at least, almost exclusively carried on in the intestinal canal, and the symptoms which are exhibited by the rest of the body are all referable to physical effects of this chemical process. It is carried on in its earliest stages upon the layer of mucus and epithelium which covers the cells of the intestinal membrane. Whatever kind or quantity of adventitious matter the cholera infection, the very particles which import the disease, may bring, this mass of mucus and epithelium breaks up according to certain conditions inherent in its chemical constitution. As its chemical constitution approaches that of the albuminous bodies, the products of its chemical decomposition are also similar to those furnished by the albuminous bodies. And as the products of decomposition contained in rice-water are probably not derived from mucin only, but also from the albumin which transudes into the cavity of the intestine in consequence of the destruction of its epithelium, their examination becomes a much more complicated problem than is at first sight apparent.

In the course of some researches on cholera which I carried

on during the epidemic of 1866 (*Ninth Rep. of the Med. Off. Privy Council*, 1866, Appendix N<sup>r</sup>. 10, p. 458), I had subjected rice-water to dialysis, and found that the dialysate from the alkaline rice-water had an acid reaction; it yielded on further analysis crystallised leucin, an oily substance soluble in water, and giving a peculiar pink reaction with nitric acid, probably indol, butyric acid, and inorganic salts in considerable quantity, but no urea.

As the rice-water continued to decompose during dialysis, or during any operation, even filtration, with evolution of gas, this process was observed as far as opportunities permitted, and it was found that the gas evolved consisted at first almost entirely of nitrogen, with only little carbonic acid; gradually the latter increased, while the nitrogen evolved decreased, until at last the carbonic acid was almost double the volume of the nitrogen. 400 c.c. of rice-water in a suitable flask and apparatus emitted the following amounts and kinds of gas into a measuring tube over mercury.

		Mixed gases	Nitrogen	Carbonic acid
First twenty-four hours		75.25 c.c.	99 per cent.	1 per cent.
Second	„	51.00 „	52 „	48 „
Third	„	100.00 „	37.5 „	62.5 „

On the fourth day some hydrogen appeared in the tube; on the fifth day the hydrogen continued to be evolved, but carbonic acid still increased relatively to the other gases. On the sixth day the hydrogen had ceased to appear, and the gas consisted almost entirely of carbonic acid, with but very little nitrogen. Soon after this point had been reached the evolution of gas ceased almost entirely, and the liquid presented a feeble somewhat modified hemochrome spectrum.

By other reactions the presence of albumin, and peculiar albuminous bodies, was proved; and I enumerated the microscopical and chemical ingredients of rice-water isolated as follows: Vibriones, cells from the surface of the intestine, granular *débris* of cells, mucin, modified hemochrome, albumin, albuminous body giving rose-pink reaction, and another one, diffusible, and giving red reaction with nitric acid, probably indol, butyric acid, acetic acid, ammonia, inorganic salts.

*The inorganic constituents of cholera rice-water* were examined some years ago in the Pathological Institute, with the following results, here published for the first time.

The liquid with some slimy deposit, was evaporated to dryness. It left a blackish deliquescent mass, which when dry at  $100^{\circ}$  weighed 82.5 grms. This was ignited in a platinum dish; it gave out a horrible smell, much vapour, and after incineration left a white fusible ash, weighing 7.8440 grms. This ash was analysed as follows.

1.6930 gm. were treated with nitric acid, and left 0.0465 gm. or 2.74 per cent. of residue insoluble in the acid. This residue contained silica, lime, and sulphuric acid, but no fluorine. 1.448 gm. were dissolved in nitric acid, the insoluble residue was filtered off, and the filtrate treated with silver nitrate. There were obtained 1.1420 gm. silver chloride = 19.49 per cent. of ash chlorine.

The phosphoric acid in the filtrate was removed by silver oxide etc., and the lime precipitated as oxalate gave 0.1530 gm. calcic carbonate = 4.22 per cent. of ash calcium.

0.714 gm. of ash were dissolved in hydrochloric acid and the insoluble residue was filtered off. The sulphuric acid precipitated by baryum chloride etc. gave 0.1650 gm. baryum sulphate or 7.93 per cent. of ash sulphuric acid.

The filtrate, by treatment with ammonia and its carbonate, gave ( $\alpha$ ) a precipitate consisting of baryum carbonate and calcium phosphate etc.; this was dissolved in hydrochloric acid; sulphuric acid and alcohol were then added and precipitated sulphates of baryum and calcium filtered off; the filtrate was treated with ammonia, ammoniacal chloride, and sodic phosphate, and gave 0.2850 gm. magnesian pyrophosphate, equal to 8.62 per cent. of ash of magnesium.

( $\beta$ ) The filtrate from the precipitate  $\alpha$  obtained by ammonia etc. was evaporated to dryness in a platinum dish, ignited and weighed 0.6208 gm. Of this a small portion remained insoluble in water, and was removed by the filter. The soluble part was again evaporated to dryness and heated and found to weigh 0.5643 gm.

In this the potassium was estimated by platinum chloride: 0.7675 gm. of the double salt showed the presence in the ash



of 17·19 per cent. potassium. The solution containing excess of platinum etc. was treated with hydrothion ( $\text{H}_2\text{S}$ ) and the filtrate evaporated to dryness, ignited and weighed; 0·2850 grm. indicated the presence in the ash of 15·68 per cent. of sodium.

*Synopsis of results.*

Total quantity of rice-water discharge = 2 litres.

Total dry residue = 82·5 grms.

Total ash = 7·8440 grms.

Composition of the ash in 100 parts:—

Sulphuric acid ( $\text{SO}_3$ )	. . . . .	7·93
Chlorine (Cl)	. . . . .	19·49
Phosphoric acid ( $\text{P}_2\text{O}_5$ )	. . . . .	23·16
Calcium (Ca)	. . . . .	4·22
Magnesium (Mg)	. . . . .	8·63
Potassium (K)	. . . . .	17·19
Sodium (Na)	. . . . .	15·68
Iron (Fe)	. . . . .	a trace
Silica and trace of gypsum, insoluble	. . . . .	2·74
Loss in operations	. . . . .	0·96
Total .		100·00

The prevalence of potassium over sodium in this ash shows conclusively that the liquid in which it is observed is not derived to any large extent from the blood, in other words is not a serous discharge. For in blood-serum the sodium salts are always larger in quantity than the potassium salts. In the juices of muscle, on the other hand, the potassium salts are always larger in quantity than the sodium salts. A liquid, like the rice-water discharge examined in the foregoing, could therefore be derived in part at least, and perhaps to a large extent, from the muscles. At once we are led to think of the symptoms exhibited by the muscles in the cholera process, and find them to be loss of heat, loss of size, cramps and consequent pain. Seeing that there is no obstruction to either respiration or circulation in the cholera patient, we cannot ascribe the great loss of body-heat to a mere diminution of natural oxydation, but are constrained to assume that there is an action

going on in the cholera-body by which heat becomes latent. This action may reasonably be supposed to be the liquefaction of a part of the colloid constituents of the muscles. For every body, which passes from the solid into the liquid state, by whatever cause, makes a quantity of heat latent, and takes that heat from its surroundings whatever they may be. These liquefied muscular constituents diffuse into the blood, by necessity, as the blood has been concentrated by diffusion of its serum into the intestinal canal; and from the blood they are secreted and diffused by pathic exudation into the intestinal canal. It is thus that we find in the rice-water discharge mineral constituents, the proportions of the ingredients of which correspond only with those in which the same mineral ingredients are present in muscular tissue.

The potential nature of the rapid sinking of animal heat in the body affected by cholera is so striking that it has never escaped even popular observation; it is owing to this potential nature of the loss of heat that a cholera-patient either cannot be warmed at all by artificial means, or becomes much worse from the moment that the artificial heat supply takes effect upon him. His comfort is for a time increased by the relaxation of cramps, but he loses more plastic material, and has less chance of recovery.

The value of this hypothesis is not diminished by the consideration, that some hold cholera to be a heat-making process like other pyrexias not complicated with the state or stage of collapse. Only two or three cases out of, say, a thousand cases examined have ever shown increased temperature of the body during the state of collapse, and those only, or principally, in the cavities of the trunk. But it is evident that these higher temperatures occurring in highly exceptional cases can be explained just like the increased temperature of moribund persons, by a local accumulation of heat, in consequence of diminished circulating blood-supply, from contraction of blood-vessels—*asphyxia* truly so-called. But even if they could not be thus explained, they are exceptions, and are cases in which the state of collapse and of pyrexia of reaction so to say overlap each other, and in which, if they were attended by rice-water discharges, the fluidification of colloid matters, and the consequent

loss of heat, must have taken place all the same; of physical necessity, however the loss of heat was masked by a concurrent pyrexia in special protoplasmic regions not in contact with large masses of muscular tissue.

This hypothesis of the occurrence of dehydration of colloid tissue in cholera, and consequent potential sinking of temperature, arises out of the results of the examination of the mineral ingredients of rice-water discharges as above stated. The evidence is derived from materials being portions of those obtained from about twenty cases, and is therefore representative of more than exceptions. The hypothesis is greatly supported by the symptoms of cholera-collapse, and these in their turn are explicable upon the basis of the hypothesis, and have not yet found any other interpretation that could be said to be in harmony with fundamental physical laws.

## XVIII.

*EXPERIMENTS ON ANIMALS FOR ASCERTAINING THE  
STATE OF GREATEST INFECTIOUSNESS OF FERMENT-  
ING CHOLERA POISON. (From the Pathological Institute.)*

It had been asserted, and apparently on good grounds, that the specific morbid secretion ejected by cholera patients had no infective properties immediately after emission, but acquired such properties, or the power of giving cholera to fresh subjects, only after a period of decomposition lasting during from 5 to 15 days. This belief was raised to a high degree of probability by the experiments of Thiersch on the infection of white mice with fermenting cholera poison. With a view of obtaining some personal information on this subject, I imitated, in 1866, in the best manner time and opportunities permitted, the experiments of Thiersch. As no account of these experiments has ever been published, and as they are an element of consideration in the admeasuring of the significance of experiments to which the surviving animals were subjected at a later period, I here give an abstract of my records.

The matter to be tested for its infectiousness was given to the animals in the following manner. Strips of bibulous paper an inch wide were dipped into the liquid, and dried in air. It was ascertained that such paper retained only a very small amount of solid matter after drying. Of such papers an inch in length, representing a square inch, was cut off, cut small, and mixed with the food of the animals in such a manner that they had either to eat or to chew some of the paper. In a few cases only, to be specially mentioned, was a minute quantity of liquid diseased matter mixed directly with the food. The white mice employed were 51 in number, distributed in couples of a male and female each in 25 cages, one cage containing a single mouse.



*Particular history of each cage. N<sup>r</sup>. 1. Two mice.*—On Aug. 10 they were fed with one square inch of paper once dipped into rice-water taken from jejunum of E. W. Aug. 17, feeding repeated with same paper; Aug. 18, ditto; Aug. 19, ditto; Aug. 21, ditto. On Aug. 27 they were fed with 6 drops of liquid from small intestine of H., who died Aug. 24; Aug. 28, ditto; Aug. 29, ditto.

*N<sup>r</sup>. 2. Two mice.*—On Aug. 11 they were fed with 1 square inch of paper soaked with rice-water contents of ileum of E. W., dipped Aug. 10. Aug. 17, feeding repeated with same paper. Aug. 18, ditto; Aug. 19, ditto; Aug. 21, ditto.

On Aug. 27, 28, and 29, fed as N<sup>r</sup>. 1.

*N<sup>r</sup>. 3. Two mice.*—On Aug. 11 they were fed with 1 square inch of paper dipped Aug. 10 in contents of colon of E. W. Aug. 19, feeding with same paper repeated. Aug. 18, 19, and 21, ditto.

*N<sup>r</sup>. 4. Two mice.*—Aug. 11, 1 square inch of paper dipped Aug. 11 in contents of jejunum of E. W. Repeated Aug. 17 with same paper. Aug. 18, 19, and 21, ditto. On Aug. 27, 28, and 29, they were fed as N<sup>r</sup>. 3.

*N<sup>r</sup>. 5. Two mice.*—Aug. 11, 1 square inch, dipped Aug. 11, ileum of E. W. Aug. 17, feeding repeated with same paper. Aug. 18, 19, 21, ditto. On 22nd they were apparently well, but one was found dead on morning of Aug. 23.

The female mouse had the right auricle of the heart very full of clotted blood, as also both cavæ. The lungs were congested in patches. The liver contained a large tænia-like *cysticercus fasciolaris*; which had caused the gall-bladder to atrophy. The liver appeared otherwise normal. The stomach was distended with starchy matter, and some cylinder cells. The small intestines were much distended with liquid of a pinkish brown colour, that in the upper part of the bowel being frothy. It consisted of free blood-corpuscles, many pale pus-like cells of various sizes, a moderate number of cylindrical epithelial cells, with a few portions of villi retaining their shape. There was also a very large number of peculiar oval nucleated bodies, but no starch was visible. The large intestine contained formed fæces, presenting no anomaly under the microscope.

The he-mouse had the right auricle of the heart distended

with fluid reddish blood, the lungs congested throughout, the liver pale in patches, but dark behind and at circumference, the gall-bladder empty. The stomach was distended with starchy matter, which was whitish at the cardiac, slightly brown towards the pyloric end. The duodenum and upper part of small intestine were extremely distended with air; the lower part contained a brownish semi-fluid substance, which under the microscope showed abundance of columnar epithelial cells, several entire villi, and a large quantity of granular epithelium and several blood-corpuscles. The cæcum was loaded with solid faecal matter, and the colon considerably distended with air.

*N<sup>r</sup>. 6. Two mice.*—On Aug. 11 were fed upon one square inch of paper soaked with contents of colon of E. W. Aug. 17, feeding with same paper repeated; 18th, ditto; 19th and 21st, ditto; on 22nd both were apparently well, but were dead on Aug. 23. The post-mortem appearances in both mice were very similar. The intestines were much distended with gas, but there was not much fluid present. Under the microscope the contents showed a large quantity of free epithelium of the intestine. The large intestine contained fæces of normal size and composition, but no epithelium. The stomach was full of farinaceous matter.

*N<sup>r</sup>. 7. Two mice.*—One square inch of paper dipped in contents of jejunum of E. W. on Aug. 12; was fed the same day. Aug. 19, feeding repeated; ditto, 21st, 22nd, and 23rd. On Aug. 27, 28, and 29 they were fed as *N<sup>r</sup>. 4*.

*N<sup>r</sup>. 8. Two mice.*—One square inch, dipped Aug. 12, ileum, E. W. Aug. 19, 21, 22, and 23, feeding repeated. On 27th and 28th fed with six drops of fluid from colon of H. (Died Aug. 24.)

*N<sup>r</sup>. 9. Two mice.*—They were not quite full-grown animals. Fed on Aug. 12 upon one square inch of paper dipped same day in colon contents of E. W. Aug. 19, 21, 22, and 23, feeding repeated. On 27th and 28th they were fed as *N<sup>r</sup>. 8*, and on morning of September 3 were found dead, much contracted, with their bellies distended, and the evidence of diarrhœa round the roots of tails. Few shaped fæces in cage.

*N<sup>r</sup>. 10. Two mice.*—They got one square inch of paper, dipped Aug. 13 in contents of jejunum of E. W. In the evening

of Aug. 15 one of the mice was noticed to have considerable difficulty in walking; the back was bent upwards, and the hind legs spread out on each side, and somewhat dragged after the body in walking. On the cotton wool was a quantity of bloody mucus, and under the tail of the affected mouse was the same substance, leaving no doubt as to the origin of the matter which was about. The temperature of the affected mouse was notably lower than that of the non-affected animal. In the morning of Aug. 16 the animal was worse. Its skin was colder than that of the healthy mouse. A large quantity of the bloody evacuation was lying about; the back was arched, there was more difficulty in progression, the fur was staring. It was put in a separate cage, and died two hours later. The lungs were very pale, but got much redder after exposure to the air. The heart contained much blood, the right side more than the left. The small intestine contained bile-stained matter, especially in its upper part, of slimy consistence. The large intestine was full of slimy blood-stained matter. The contents of the small intestine under the microscope showed remnants of food and coverings of villi; those of the large intestine a few scattered blood-corpuscles and a great many cells having somewhat the appearance of pus, but no columnar epithelial cells.

On Aug. 19 the feeding of the remaining mouse was repeated. Ditto on Aug. 21, 22, 23. On Aug. 27 and 28 it was fed as N<sup>r</sup>. 9.

N<sup>r</sup>. 11. *Two mice*.—One square inch, ileum, E. W. Dipped Aug. 13. On Aug. 15 at 8 p.m. one of the mice was found in the inner box apparently dead. It was bent up, stiff, but still warm. There was no bloody matter in the cage or on the mouse. The mouse was placed on the outside of the cage for further observation, but on the 16th in the morning it had disappeared. The other mouse appeared healthy. With this animal the feeding was repeated on Aug. 19, 21, 22, and 23. On Aug. 27 and 28 it was fed as N<sup>r</sup>. 10.

N<sup>r</sup>. 12. *Two mice*.—One square inch, colon E. W., Aug. 13. Feeding repeated on Aug. 19, 21, 22, 23. On Aug. 27 and 28, fed as N<sup>r</sup>. 11.

N<sup>r</sup>. 13. *Two mice*.—One square inch jejunum, E. W., Aug. 14. Feeding repeated Aug. 19, 21, 22, and 23. On Aug. 28

these animals were fed with six drops of rice-water taken from small intestine of S. forty-eight hours after the death of the patient. The same repeated on Aug. 29.

*N<sup>r</sup>. 14. Two mice.*—One square inch, ileum, E. W., Aug. 14. Aug. 19, 21, 22, and 23, feeding repeated. On Aug. 28 and 29, fed as *N<sup>r</sup>. 13*.

*N<sup>r</sup>. 15. Two mice.*—One square inch, colon, E. W., Aug. 14. Aug. 19, 21, 22, and 23, feeding repeated. On Aug. 28 and 29, fed as *N<sup>r</sup>. 14*.

*N<sup>r</sup>. 16. Two mice.*—One square inch, jejunum, E. W., dipped Aug. 15. Feeding repeated Aug. 19, 21, 22, 23. On Aug. 28 and 29, fed as *N<sup>r</sup>. 15*.

*N<sup>r</sup>. 17. Two mice.*—One square inch, ileum, E. W., dipped Aug. 15. Aug. 19, 21, 22, and 23, feeding repeated. On Aug. 28 and 29, fed as *No. 16*. These two mice were found dead on the morning of Sept. 3. There were some cake-shaped fæces. The male had fæcal incrustations round anus and root of tail. The female was covered on its belly and side with fluid matter.

*N<sup>r</sup>. 18. Two mice.*—One square inch, colon, E. W., dipped Aug. 15. Feeding repeated Aug. 19, 21, 22, and 23. On 28 and 29, fed with six drops of rice-water stool passed by M. A. T. on Aug. 16.

*N<sup>r</sup>. 19. Two mice.*—One square inch, jejunum, E. W., dipped Aug. 13, and dried; fed Aug. 16. This was the same paper as that which had been given to *N<sup>r</sup>. 10* mice.

*N<sup>r</sup>. 10 mice.* On Aug. 19 they were fed with bread on which six drops of mixed rice-water from a cholera patient, passed Aug. 17, had been dropped. This feeding was repeated on Aug. 21, 22, and 23. On Aug. 28 and 29, the mice were fed as *N<sup>r</sup>. 18*.

*N<sup>r</sup>. 20. Two mice.*—On Aug. 16 these mice received one square inch of paper, dipped on Aug. 13 in contents of ileum of E. W., being the same paper as that upon which mice *No. 11* were fed. On Aug. 19 they were fed in the same way as *N<sup>r</sup>. 19*, also on Aug. 21, 22, 23. On Aug. 28 and 29, fed like *N<sup>r</sup>. 19*.

*N<sup>r</sup>. 21. Two mice.*—One square inch, dipped Aug. 16, jejunum, E. W. Aug. 19, fed with one square inch more. On



Aug. 20 they were fed with sopped bread at 10 A.M. They ate some, but about half an hour later they were found both dead, and yet quite warm. There had been no purging, and their skins had not felt colder than those of healthy mice. They were left in the cage till evening, but did not revive. The white mouse was examined ten hours after death. Its abdomen was much distended, so that on opening it the intestines immediately protruded. The large intestine was moderately filled with solid contents of a greyish colour. From the small intestine, when cut across, nearly half a tablespoonful of pinkish semi-fluid matter escaped. This under the microscope appeared to consist of cells of columnar epithelium which lay sometimes in masses, of which several had the shape of distinct caps of villi. The brown and white mouse was examined thirty hours after death. Its belly was distended and its hair somewhat matted as if from wet. The lungs were completely sunk into the back of their cavity; they were congested behind, with pale patches on the anterior margins, and on exposure to air became much redder than they had been. The right auricle of the heart was much distended with dark blood. The stomach was distended by food and gas. At the cardiac end was a mass of almost pure sopped bread, in the centre a large mass of gas, and at the pyloric end a smaller quantity of semi-fluid pinkish buff-coloured matter, almost exactly like that in the duodenum. The line of demarcation between the white bread and the pinkish pyloric portion was very distinct. The pyloric part contained much starch, but the bulk of its contents was composed of cylindric epithelium, so that a retroperistaltic motion must have taken place. In the duodenum and entire small intestine there was nearly the same substance as in the pyloric end of the stomach; it consisted almost entirely of cylindric epithelium, with a number of fat-globules, but, as was shown by means of iodine, no starch. In order to prevent the admixture of any intestinal epithelium which might be detached by the mechanism of the examination, the intestine was cut across, and the contents allowed to flow out assisted by the very slightest pressure required. Some cells were thus obtained in the form of caps of villi. The large intestine contained semi-fluid faecal matter of greyish green colour in the upper part, and soft faeces in the

lower; in neither was there any notable amount of epithelium, if the intestine had not been forcibly pressed. The liver was very pale, the gall-bladder could not be detected, the urinary bladder, though empty, was more collapsed than contracted.

*N<sup>r</sup>. 22. Two mice.*—One square inch, dipped Aug. 16, ileum, E. W. Repeated Aug. 19, 21, 22, 23. On Aug. 28 and 29, fed as *N<sup>r</sup>. 20*.

*N<sup>r</sup>. 23. Two mice.*—One square inch, dipped Aug. 16, colon, E. W. Repeated Aug. 19, 21, 22, 23. On Aug. 28 and 29, fed as *N<sup>r</sup>. 22*.

*N<sup>r</sup>. 24. Two mice.*—One square inch, dipped Aug. 17, jejunum, E. W. Aug. 19, fed with bread on which six drops of fluid from jejunum of E. W. had been placed. Repeated Aug. 21 and 22. On Aug. 23 there were several loose motions in the cage of reddish-brown colour and slimy consistence; they contained a good many blood-corpuscles, and the usual materials of fæces, but no epithelium. There were also some bacteria, and numerous bodies like eggs of entozoa, of unexplained significance.

*N<sup>r</sup>. 25. Two mice.*—One square inch, dipped Aug. 17, ileum, E. W. Aug. 19, fed with bread upon which six drops of fluid from ileum of E. W. had been placed. Repeated on Aug. 21, 22, and 23.

*N<sup>r</sup>. 26. One mouse.*—One square inch, dipped Aug. 17, colon, E. W. Aug. 19, fed with bread upon which six drops of fluid from colon of E. W. had been placed. Repeated same Aug. 21, 22, and 23.

The experiments were terminated towards the end of August, and the remaining forty mice forwarded to Dr. J. B. Sanderson, who then made upon them the series of experiments which he himself has recorded.

It will be at once observed how small is the number of recovering animals compared to the number which died. Most remarkable however is the circumstance that out of the eleven deaths ten were those of pairs, of which each was living in a cage together, and that these deaths of the individuals of each couple took place almost at the same time. This points to a very definite cause of disease, a simultaneous introduction of its poison, and a great similarity in the manner in which the

animals susceptible of it reacted upon it. The cause of the immunity of other animals apparently subject to the same influences remains unexplained.

LIST OF CAGES AND ANIMALS, SHOWING CASES OF SICKNESS (\*) AND DEATH (†), AND ANIMALS NOT AFFECTED.

1. Two mice.	13. Two mice.
2. Two mice.	14. Two mice.
3. Two mice.	15. Two mice.
4. Two mice.	16. Two mice.
5. Two mice; both died, Aug. 23.††	17. Two mice; both died, Sept. 3.††
6. Two mice; both died, Aug. 23.††	18. Two mice.
7. Two mice.	19. Two mice.
8. Two mice.	20. Two mice.
9. Two young mice; both died, Sept. 3.††	21. Two mice (one brown and white); both died, Aug. 20.††
10. Two mice; one died, Aug. 16.	22. Two mice.
11. Two mice; one sick (disap- peared).*	23. Two mice.
12. Two mice.	24. Two mice; bloody diarrhœa.*
	25. Two mice.
	26. One mouse.
Total number of animals . . . . .	51
Total number of sick . . . . .	13
Total number of deaths . . . . .	11
Recovered . . . . .	2
Not affected . . . . .	38
	51

*The symptoms* exhibited by the infected mice were very uniform. They had diarrhœa, bloody diarrhœa, loss of urinary secretion; they became cold, their temperature sank; they became stiff, so as to appear dead while yet feebly living; they died, and in such positions that it was probable that spasm was one of the ultimate symptoms. Their intestines were filled with whitish matter, and their abdomens always distended. In short, there was a resemblance to human cholera which could not escape consideration. Most of the animals were pure albinos, a circumstance to be remembered, as we know, from experiments concerning the excision of the supra-renal capsules, that albinos have a physiological economy differing in some

respects, besides their peculiarities as to the chemistry of pigment, from that of other animals not albinos. In this respect it must be noted that one of the mice which died was grey and white, but as it was bred from albino stock by crossing with a rare variety it forms a singular exception to the fact that all the animals employed were albinos.

In the following seven tables the experiments are arranged in such a manner that the effects of the cholera poison taken from different parts of the intestinal canal after death become conspicuous. The seventh table alone relates to experiments with excretions from living cholera-patients.

TABLE I.—RESULTS OF FIRST SERIES OF EXPERIMENTS.

*Contents of Jejunum of E. W.; removed Aug. 10, 1866.*

Paper dipped Aug. 10, or 1st fermentation day	{ Mice N <sup>o</sup> 1. Fed Aug. 10, also Aug. 17, 18, 19, 21. No effect.
Paper dipped Aug. 11, or 2nd fermentation day	{ Mice N <sup>o</sup> 4. Fed Aug. 11, also 17, 18, 19, 21. No effect.
Paper dipped Aug. 12, or 3rd fermentation day	{ Mice N <sup>o</sup> 7. Fed Aug. 12, also 19, 20, 21, 22, 23. No effect.
Paper dipped Aug. 13, or 4th fermentation day	{ Mice N <sup>o</sup> 10. Fed Aug. 13. A mouse sick on Aug. 15. † Aug. 16. 2nd mouse fed Aug. 19, 20, 21; 2nd mouse well, 22, 23.
	{ Mice N <sup>o</sup> 19. Fed upon 4th fermentation dip, on Aug. 13, to test the case of Mice N <sup>o</sup> 10, but no effect.
Paper dipped Aug. 14, or 5th fermentation day	{ Mice N <sup>o</sup> 13. Fed Aug. 14, also 19, 20, 21, 22, 23. No effect.
Paper dipped Aug. 15, or 6th fermentation day	{ Mice N <sup>o</sup> 16. Fed Aug. 15, also 19, 20, 21, 22, 23. No effect.
Paper dipped Aug. 16, or 7th fermentation day	{ Mice N <sup>o</sup> 21. Fed Aug. 16, also Aug. 19, 20. Both died Aug. 20.††
Paper dipped Aug. 17, or 8th fermentation day	{ Mice N <sup>o</sup> 24. Fed Aug. 17; on Aug. 19, 20, 21, 22, with 6 drops, with paper of fluid 8th fermentation day. 1st mouse, bloody diarrhoea.
10th, 12th, 13th, 14th fermentation days, 6 drops of, each day	{ No effect observed.

Consequently poisonous properties appeared first on the 4th fermentation day, reached maximum on the 7th, and declined again on the 8th. The latest effect was on 4th day after feeding; the earliest may have been on 1st, probably was on 3rd day.



TABLE II.—RESULTS OF SECOND SERIES OF EXPERIMENTS.

*Contents of Ileum of E. W.; removed Aug. 10, 1866.*

Paper dipped Aug. 10, or 1st fermentation day	{ Mice N <sup>o</sup> . 2. Fed Aug. 10, also 17, 18, 19, 21. No effect.
Paper dipped Aug. 11, or 2nd fermentation day	{ Mice N <sup>o</sup> . 5. Fed Aug. 11, also 17, 18, 19, 21. Both dead, Aug. 23.††
Paper dipped Aug. 12, or 3rd fermentation day	{ Mice N <sup>o</sup> . 8. Fed Aug. 12, also 19, 21, 22, 23. No effect.
Paper dipped Aug. 13, or 4th fermentation day	{ Mice N <sup>o</sup> . 11. Fed Aug. 13. On 15th one mouse was apparently dead and stiff; disap- peared. The other mouse was fed on Aug. 19, 21, 22, 23. No effect. Mice N <sup>o</sup> . 20. Fed on Aug. 16, upon 4th fer- mentation day's paper, as mice N <sup>o</sup> . 11. No effect.
Paper dipped Aug. 14, or 5th fermentation day	{ Mice N <sup>o</sup> . 14. Fed Aug. 14, also 19, 21, 22, 23. No effect.
Paper dipped Aug. 15, or 6th fermentation day	{ Mice N <sup>o</sup> . 17. Fed Aug. 15, also 19, 21, 22, 23. No effect. (See however Series VI., rice-water, Aug. 28 and 29.)
Paper dipped Aug. 16, or 7th fermentation day	{ Mice N <sup>o</sup> . 22. Fed Aug. 16, also 19, 21, 22, 23. No effect.
Paper dipped Aug. 17, or 8th fermentation day	{ Mice N <sup>o</sup> . 25. Fed Aug. 17, also with 6 drops of fluid on Aug. 19, 21, 22, 23. No effect.
Also 10th, 12th, 13th, 14th fermentation days, 6 drops each day	{ No effect.

The contents of the ileum reached their maximum infectiousness on the 2nd fermentation day; this declined on the 4th, and did not afterwards appear at all.

TABLE III.—SHOWING RESULTS OF THIRD SERIES OF EXPERIMENTS.

*Contents of Colon of E. W.; removed Aug. 10, 1866.*

Paper dipped Aug. 10, or 1st fermentation day	{ Mice N <sup>o</sup> . 3. Fed Aug. 10, also 17, 18, 19, 21. No effect.
Paper dipped Aug. 11, or 2nd fermentation day	{ Mice N <sup>o</sup> . 6. Fed Aug. 11, also 17, 18, 19, 21. Aug. 23, both dead.††
Paper dipped Aug. 12, or 3rd fermentation day	{ Mice N <sup>o</sup> . 9. Fed Aug. 12, also 19, 21, 22, 23. No effect. (See however Series V., Colon, Sept. 3.)
Paper dipped Aug. 13, or 4th fermentation day	{ Mice N <sup>o</sup> . 12. Fed Aug. 13, also 19, 21, 22, 23. No effect.

Paper dipped Aug. 14, or { Mice N<sup>o</sup>. 15. Fed Aug. 14, also 19, 21, 22, 23.  
 5th fermentation day { No effect.  
 Paper dipped Aug. 15, or { Mice N<sup>o</sup>. 18. Fed Aug. 15, also 19, 21, 22, 23.  
 6th fermentation day { No effect.  
 Paper dipped Aug. 16, or { Mice N<sup>o</sup>. 23. Fed Aug. 16, also 19, 21, 22, 23.  
 7th fermentation day { No effect.  
 Paper dipped Aug. 17, or {  
 8th fermentation day; { Mice N<sup>o</sup>. 26. Fed Aug. 17, also with 6 drops  
 also 10th, 12th, 13th, { on Aug. 19, 21, 22, 23. No effect.  
 and 14th fermentation {  
 days }

The contents of the colon had infectious power only on the 2nd fermentation day, and were inert from the 3rd to the 14th fermentation day.

TABLE IV.—RESULTS OF FOURTH SERIES OF EXPERIMENTS.

*Contents of Small Intestine (H., died Aug. 24).*

Day of Month	Day of fermentation	Mice N <sup>o</sup> . 1	Mice N <sup>o</sup> . 2
August 27	3rd	} Fed upon 6 drops. No effect.	Fed upon
" 28	4th		6 drops.
" 29	5th		No effect.

Day of month	Mice N <sup>o</sup> . 3	Mice N <sup>o</sup> . 5	Mice N <sup>o</sup> . 7
August 27	} Fed upon 6 drops. No effect.	Fed upon	Fed upon
" 28		6 drops.	6 drops.
" 29		No effect.	No effect.

TABLE V.—RESULTS OF FIFTH SERIES OF EXPERIMENTS.

*Contents of Colon (H.).*

Day of month	Day of fermentation	Mice N <sup>o</sup> . 8	Mice N <sup>o</sup> . 9
August 27	3rd	{ Fed upon 6 drops. No effect.	Fed upon 6 drops.
" 28	4th		Both died, †† Sept. 3.

Day of month	Mice N <sup>o</sup> . 10	Mice N <sup>o</sup> . 11	Mice N <sup>o</sup> . 12
August 27	{ Fed upon 6 drops. No effect.	Fed upon	Fed upon
" 28		6 drops. No effect.	6 drops. No effect.

TABLE VI.—RESULTS OF SIXTH SERIES OF EXPERIMENTS.

*Rice-water from Small Intestine (S.).*

Day of month	Day of fermentation	Mice N <sup>o</sup> 13	Mice N <sup>o</sup> 14
August 28 " 29	2nd 3rd	{ Fed upon 6 drops. No effect.	Fed upon 6 drops. No effect.

Day of month	Mice N <sup>o</sup> 15	Mice N <sup>o</sup> 16	Mice N <sup>o</sup> 17
August 28 " 29	{ Fed upon 6 drops. No effect.	Fed upon 6 drops. No effect.	Fed upon 6 drops. Both died,++ Sept. 3.

The date of the death of N<sup>o</sup> 17 being the same as that of N<sup>o</sup> 9 mice suggests some cause of infection to which both were simultaneously subjected previously. Mice N<sup>o</sup> 9 had taken contents of colon, mice N<sup>o</sup> 17 of ileum of E. W., but ten days previously.

TABLE VII.—RESULTS OF SEVENTH SERIES OF EXPERIMENTS.

*Rice-water Stool from M. A. T.*

Day of month	Day of fermentation	Mice N <sup>o</sup> 18	Mice N <sup>o</sup> 19
August 28 " 29	2nd 3rd	{ Fed upon 6 drops. No effect.	Fed upon 6 drops. No effect.

Day of month	Mice N <sup>o</sup> 20	Mice N <sup>o</sup> 22	Mice N <sup>o</sup> 23
August 28 " 29	{ Fed upon 6 drops. No effect.	Fed upon 6 drops. No effect.	Fed upon 6 drops. No effect.

## XIX.

*NOTE AND EXPERIMENTS ON THE ALLEGED EXISTENCE  
IN THE BRAIN OF A BODY TERMED 'PROTAGON'  
(From the Pathological Institute.)*

SOME authors have lately revived what could have been called at one time the hypothesis of 'protagon,' and have reasserted the existence of this matter as a definite chemical entity or immediate principle in the brain. The result of some new analyses is an empirical formula,  $C_{160}H_{308}N_5PO_{35}$ , which differs from the earlier formula  $C_{116}H_{241}N_4PO_{22}$  by a quantity of no less than  $C_{54}H_{67}NO_{13}$ . In these analyses also much less hydrogen in proportion to the carbon was found than in the earlier ones, although they refer to virtually the same substance. The processes by which the operators on 'protagon' obtained their matter, are essentially the same as those which were employed by Vauquelin, Couerbe, Frémy, Gobley, Bibra, Thomson and others, for the extraction from brain of a matter which had the same percentic composition as 'protagon' with regard to all elements, phosphorus alone excepted, as is evident from the following comparison of the analytical data.

	Frémy's cerebric acid	Gobley's cerebrin	Bibra's cerebric acid	Thomson's cerebric acid	Liebreich's protagon	Gamgee and another's product
C	66.7	66.85	66.78	67.04	66.74	66.39
H	10.6	10.82	10.65	10.85	11.74	10.96
N	2.3	2.29	2.51	2.24	2.80	2.39
P	0.9	0.43	0.52	0.46	1.23	1.06
O	19.5	19.61	19.52	19.41	17.49	19.47

'Protagon' was therefore by no means a needful discovery, and if this substance were really a definite educt, it would be absolutely the same as Frémy's cerebric acid. This fact was



somewhat obscured by the circumstance that authors on 'protagon' ignored the existence of previous researches, and complicated the otherwise well-known processes of preparation of the white matter from brain by fanciful additions; and these amplifications were so unmeaning, that by their inventors themselves they were in ultimate proceedings entirely abandoned.

Now in spite of this unquestionable priority of Frémy, whose name the authors who treat of 'protagon' never mention, and whose work they have unquestionably either not studied or not heeded, they state that what they believe or pretend to be their discovery concerned the first definitively ascertained specific constituent of the brain; they boast that their own investigations had been carried out with all the necessary care and even minuteness, and that they had ascertained not merely the amount of phosphorus, but also that of all the other elements contained in their production.

Frémy had shown that the body to which he gave the name of cerebrie acid, was often combined with phosphate of lime, or with soda, and had to be freed from these by treatment with a little sulphuric acid in alcoholic solution. It was this circumstance which induced him to term the body which he had isolated an acid. In consequence of these observations of Frémy's I examined all educts from the brain which I have described in my researches for mineral ingredients, and found that when they are isolated as far as neutral solvents will isolate them, *they all contain considerable quantities of all, or some, or one of the following bodies: ammonium, sodium, potassium, calcium, iron, copper, calcic and magnesian phosphates.* (See my 'Researches on the Chemical Constitution of the Brain,' *Reports of the Medical Officer of the Privy Council and Local Government Board*, New Series, N<sup>o</sup>. III. 1874, p. 129; also 'Further Researches on the Chemical Constitution of the Brain,' *Reports*, etc., New Series, N<sup>o</sup>. VIII. 1876, p. 131.) I have given complete quantitative analyses of these bodies as contained in the phosphorised principles discovered by me, namely, *kephalin* and *myelin*. In one experiment in which the bases were extracted by hydrochloric acid, as much as seven grammes of alkaline chlorides were obtained.

These inquiries were already several years ago extended to

the product termed 'protagon,' and this too was found to contain inorganic constituents irremovable by recrystallisation from alcohol, however frequently repeated. It was found more particularly that 'protagon' and the bodies into which it can be separated, according to my researches, always retain considerable quantities of *potassium* in combination. As the quantity of inorganic ingredients in 'protagon' had never been estimated, I prepared a specimen of this matter, and on analysis found it to contain the better part of one per cent. of inorganic incombustible matter, phosphoric acid not included, and in this no less than 0.76 *per cent. of the 'protagon' of potassium*. This same 'protagon' contained 1.057 per cent. of phosphorus, and ascribing to it (hypothetically) the mean amount of carbon, hydrogen and nitrogen found by the latest operators, it would be necessary to consider and rename 'protagon' as the potassium salt (mainly) of an acid (in justice to Frémy to be termed cerebrie acid), and to give it the formula no longer empirical, but compulsory, if 'protagon' were a definite unitary educt of  $C_{284}H_{548}N_9P_2KO_{60}$ . If this hypothesis should fail to account for the data, the following conclusion becomes unavoidable, namely, that it is not the fact that any easy process such as that described for the isolation of 'protagon' yields a product in a state of purity, but that the 'protagon' thus far obtained, apart from the question whether or not it is a mixture of organic compounds, is yet very impure.

The special test experiment (*Exp. I.*) alluded to in the foregoing, gave results of permanent interest in the consideration of the general question, which deserve to be here recorded. Twelve ox-brains were extracted with spirit of 85 per cent. strength at 45°. All the extracts were cooled to 0° and allowed to stand over night. The deposited white matter was collected and exhausted with ether.

It was now subjected to the process termed recrystallisation, and thereby split up into the following eight different products.

A. Extraction by five litres of spirit at 45° gave a solution, which on standing during 16 hours at 17°, without being cooled to 0°, deposited a white matter, the principal bulk of the 'protagon,' weighing, dry, 30.9 grms. From this large volumes of

ether extracted nearly one grm. of matter (product N<sup>r</sup>. I. 1) containing 0.52 per cent. of phosphorus. The remaining 30 grms. of protagon once recrystallised (product N I. 2) contained 1.057 per cent. of phosphorus and 0.76 per cent. of potassium.

The spirituous mother liquor, which had deposited the foregoing white matter yielding products N I. 1, and I. 2, on standing during five days at an average temperature of 17°, deposited a voluminous mass of matter which when dry, weighed 3 grms. and contained 1.13 per cent. P, and 0.22 per cent. K (product N<sup>r</sup>. I. 3). The filtrate from this was now placed in water, kept cold by ice during 18 hours, and deposited a matter (product N<sup>r</sup>. I. 4) which when dry weighed 5 grms. and contained 2.02 per cent. P and 0.39 per cent. K. The spirituous filtrate from this fourth product gave precipitates with cadmic chloride, platinic chloride, and lead acetate, and after distillation of the spirit and drying left 10 grms. of a yellowish, highly hygroscopic residue containing 2.91 per cent. of P and traces of K (product N<sup>r</sup>. I. 5).

B. The white matter from which five litres of spirit had extracted the mixture of the five several products described in the foregoing was extracted twice more with on the whole about four litres of spirit at 45°. The solutions deposited on cooling and standing 8 grms. dry, of a white matter containing 0.76 per cent. P and 1.44 per cent. K (product N<sup>r</sup>. I. 6).

C. A considerable quantity of white matter, which had been dissolved in the first spirit at 45°, was left undissolved by spirit at 45° even on very long digestion. It weighed, dry, 13 grms., and contained large rhombic crystals, resembling cholesterin, but insoluble in either ether or alcohol, and therefore not being cholesterin. Ether extracted from these 13 grms. of residue 0.4508 grm. of a matter which contained 0.41 per cent. P (product N<sup>r</sup>. I. 7). The remainder of the matter insoluble in alcohol at 45° and in ether, weighing more than 10 grms., is product N<sup>r</sup>. I. 8.

The following list shows the quantities of the eight products obtained, and their percentages of phosphorus and potassium.

Numbers of Products		Quantity	Phosphorus	Potassium
		Grammes	Per cent.	Per cent.
N <sup>r</sup> . I.	1	1.0	0.52	?
"	2	30.0	1.057	0.76
"	3	3.0	1.13	0.22
"	4	5.0	2.02	0.39
"	5	10.0	2.91	traces
"	6	8.0	0.76	1.44
"	7	0.4508	0.41	—
"	8	12.0	—	—
"	8	} 10.0	0.108	traces
recrystallised				

Here it must be pointed out that all writers on 'protagon' have left these products all but N<sup>r</sup>. 2 entirely out of consideration, just as they have ignored a large quantity of white matter which is not extracted at all from brain-pulp by spirit at 45°, but requires boiling spirit for solution.

The so-called 'protagon' forms only thirty parts out of nearly seventy parts of what remained of the white matter extracted by spirit at 45° from twelve ox-brains after exhaustion with large volumes of ether to such a degree that two litres of ether digested with the matter during six hours left only one decigramme of residue.

Attention may be directed to the fact that in the different products phosphorus and potassium do apparently observe an opposite ratio, potassium sinking when phosphorus rises, and phosphorus sinking when potassium rises in quantity. The further investigation of these relations will no doubt lead to important and interesting results, but they are foreign to our present purpose.

D. *Separation of 'protagon' into bodies differing in composition by absolute alcohol at 45°.*—The product N<sup>r</sup>. I. 2 above described, corresponding to 'protagon once recrystallised' and containing 0.76 per cent of potassium was now *treated with 1½ litres absolute alcohol* during 20 hours at 45°. There remained 7.6 grms. undissolved, and this (product N<sup>r</sup>. I. 9) contained 0.94 per cent. P and 1.67 per cent. K. The deposit from the solution on cooling during 3 hours at 20°, corresponding to 'protagon twice recrystallised' weighed 8 grms. (product N<sup>r</sup>. I. 10) and contained 0.83 per cent. P with 1.41 per cent K.



The solution filtered from this on standing during three days at a mean temperature of 21° made a further deposit which weighed 4.3 grms. (product N<sup>r</sup>. I. 11) and contained 0.26 per cent. P and 0.22 per cent. K. The mother liquor on distillation yielded 5.9 grms of matter (product N<sup>r</sup>. I. 12) containing 1.77 per cent. P and 0.14 per cent. K.

*Synopsis of the products obtained from 'protagon' by resolution in absolute alcohol at 45° etc.*

Numbers of products	Quantity	Phosphorus	Potassium
	Grammes	Per cent.	Per cent.
N <sup>r</sup> . I. 9	7.6	0.94	1.67
„ 10	8.0	0.83	1.41
„ 11	4.3	0.26	0.22
„ 12	5.9	1.77	0.14

The matter insoluble in alcohol at 45, weighing more than 12 grms. (product N<sup>r</sup>. I. 8) was found to dissolve entirely in 300 c.c. of boiling spirit, leaving albumin and paper fibre, together 1 gram. undissolved. On cooling a deposit rapidly separated which weighed 10 grms. and contained 0.108 per cent. P and mere traces of K. All these twelve deposits contained the usual phosphorised and cerebrin bodies, namely myelin, phrenosin, and kersin; the mother liquor deposited kersin like all other mother liquors of the cerebrin bodies (including the boiled ones, as we shall presently show), and gave copious myelin precipitates with cadmic and platinic chloride.

In another experiment (*Exp. II.*), six ox-brains were extracted with large volumes of spirit of 85 per cent. strength at 45°. The solution was allowed to stand at 16° but was not cooled to 0°. The white matter was isolated and exhausted with ether. It weighed dry 25 grms. and contained 0.87 per cent. P. The whole of this was now *boiled in spirit of 85 per cent.*, in which it was completely soluble. It was again deposited on cooling, and the deposit now contained 0.84 per cent. of P. The P had therefore been diminished by one recrystallisation. This matter was now differentiated into four fractions by treatment with spirit of 85 per cent. at 45°.

A. Two litres of spirit digested with the 'protagon' gave on cooling a deposit (product N<sup>r</sup>. II. 1), which weighed dry

11 grms. and contained 0.91 per cent. of P and 0.88 per cent. of K. The mother liquor left 2.3 grms. of yellowish white matter (product N<sup>r</sup> II. 2), which contained 1.83 per cent. of P and 0.87 per cent. of K.

*B.* One litre of spirit was digested with the matter which spirit in *A* had left undissolved for 18 hours at 45°, and gave on cooling a deposit, weighing 3.9 grms. (product N<sup>r</sup> II. 3) containing 0.74 per cent. P and 1.04 per cent. K.

*C.* The matter left undissolved by spirit in *B* was again digested at 45° with 1 litre of spirit for 6 hours. On cooling the solution gave a deposit, weighing 1.6 gm. (product N<sup>r</sup> II. 4) containing 0.46 per cent. P and 1.23 per cent. K.

*D.* There were left undissolved by these three extractions with spirit at 45°, 4.4 grms. of matter (product N<sup>r</sup> II. 5), which contained 0.12 per cent. P and 0.54 per cent. K.

The mother liquors of *B* and *C* contained very little matter. All the fractions summed up, including parts used for analysis, amount to 23 grms. out of 25 grms. originally operated on, so that the amount left in the mother liquors and lost in operations could not amount to more than 2 grms.

*Synopsis of the fractions obtained in Exp. II. by extracting 'protagon' with spirit at 45°.*

Numbers of fractions		Quantity	Phosphorus	Potassium
		Grammes	Per cent.	Per cent.
N <sup>r</sup> II.	1	11.0	0.91	0.88
"	2	2.3	1.83	0.87
"	3	3.9	0.74	1.04
"	4	1.6	0.46	1.23
"	5	4.4	0.12	0.54

Here we observe again, as in Exp. I., the peculiarly shifting proportions between phosphorus and potassium. Not a single fraction is free from potassium, and no two fractions contain the same amount of either phosphorus or potassium. All the fractions are mixtures of the same kind, as the fractions obtained in Exp. I. at the same stages; in the corresponding fractions of both experiments P and K observe the same relations, and are present in almost the same quantity; each element at all events observes the same curve in both experiments. The boiling in

spirit had therefore not effected any greater difference than the fractional solution at 45° and precipitations in Exp. I. The appearance of the fractions was the same as that of the corresponding fractions from Exp. I., and on further testing and differentiation they yielded the well-known constituents just as the products from Exp. I.

In a third experiment (*Exp. III.*), a quantity of white matter from the human subject was exhausted with ether, and the product, about 60 grms., digested with 4 litres of spirit at 45° for 18 hours. There was left undissolved 4·5 grms. of matter (product N<sup>r</sup>. III. 1), which after solution in boiling spirit and filtration from some albumin contained 0·46 per cent. P and 0·26 per cent. K. The solution was cooled rapidly to 21°, and allowed to deposit during four hours; the deposit collected and dried weighed 32 grms. (product N<sup>r</sup>. III. 2), and contained 0·87 per cent. P and 0·41 per cent. K. The filtrate from this principal precipitate was now put into ice-cold water for 18 hours, and gave a deposit which after isolation was white and waxy, weighed 8·6 grms. (product N<sup>r</sup>. III. 3), and contained 1·69 per cent. P and 0·17 per cent. K. The mother-liquor on distillation left nearly 12 grms. of a yellowish waxy hygroscopic body (product N<sup>r</sup>. III. 4), containing 2·85 per cent. P and 0·09 per cent. K.

*Synopsis of the fractions obtained by extracting human  
'protagon' with spirit at 45°.*

Numbers of fractions	Quantity	Phosphorus	Potassium
	Grammes	Per cent.	Per cent.
N <sup>r</sup> . III. 1	4·5	0·46	0·26
" 2	32·0	0·87	0·41
" 3	8·6	1·69	0·17
" 4	12·0	2·85	0·09

The state of aggregation of the particles of 'protagon' has been described as crystallised, mostly in microscopic rosettes, but it is admitted that they differ somewhat according to the degree of concentration of the solution from which they are deposited. I inspected microscopically the forms obtained by the recrystallisation of the product N<sup>r</sup>. I. 2, which contained 1·05 per cent. of phosphorus, and 0·76 per cent. of potassium.

I at once perceived the white rosettes of phrenosin, the opaque balls with radiary arrangement of needles of cerebrinic acid, the yellowish balls with concentric layers of myelin, the amorphous particles of another myelin, and over all, the wavy long filaments of the blue iridescent kerasin.

The bodies named can be separated out of the 'protagon' by the processes which I have described; the myelins mainly by combination with platinic chloride, or cadmic chloride or sulphide, or lead oxide. The myelins are not more soluble in ether than the cerebrins, and thereby differ and are easily separated from the lecithins, which are very soluble in cold ether, and cold alcohol or even spirit; the lecithin platinic chloride is soluble in ether, while myelin platinic chloride is insoluble in ether.

The cerebrins are obtained free from phosphorus mainly by very frequent resolution in hot absolute alcohol. They cannot be separated from each other by this process alone; lead acetate and a little ammonia are required to make cerebrinic acid insoluble in hot spirit; phrenosin and kerasin do not permanently combine with lead, they are separated from each other by fractional recrystallisation from suitable volumes of absolute alcohol; phrenosin is deposited earlier at a temperature above 28°, kerasin later at temperatures below 26°, and on long standing. But even so they cannot be liberated entirely from inorganic matters, particularly potassium, and a trace of calcic phosphate; even the lead acetate treatment above described removes only a part, though the greater part of these bases, as acetates: the last traces have to be extracted by cautious treatment with mineral acid in alcoholic solution.

The foregoing data enable us to attribute their proper value to the series of operations by which the advocates of 'protagon' have brought about the concordance of their analyses. The potassium, which though present in such quantity that if 'protagon' were a unitary body its atomic weight would thereby be fixed they have not found, is calculated as oxygen; the phosphate of lime which they have not extracted is made to increase the protagonal phosphorus; the mixture of the myelins, which they have not extracted, and which they are unable to diagnose, is adjusted by solvents to a convenient quantity and made to



represent the constituent phosphorus of 'protagon'; the cerebrins are not separated from each other, and, by a process called slow crystallisation, are made to deposit in such a manner that they must be covered by the only one amongst them which is distinctly crystalline, and is the one moreover which is present in the smallest quantity.

The uniform chemical composition of the brain greatly favours the obtaining from it by the aid of processes nearly akin to trimming, of extracts of uniform composition; this uniformity can be greatly aided by limitations of the quantities of materials operated upon, and of the quantities and strengths of the solvents, and by careful observance of these limitations, preparations are obtained which present a delusive appearance of definiteness. But this delusion could only be persevered in by persons who are not in the habit of subjecting their products to tests of purity, and who are not acquainted with the necessity which is imposed upon every conscientious inquirer of questioning his products and conclusions in a sense adverse to his hypothesis.

It follows from the foregoing that the doctrine of 'protagon' has been properly rejected by all physiological chemists who know their business.

## XX.

ON THE COLOURING MATTERS OF BILE AND OF GALL-STONES, THEIR COMPOUNDS, DERIVATES, CHEMICAL AND SPECTROSCOPIC PHENOMENA. (*Consolidated Account of Researches. From the Pathological Institute.*)

*Mode of obtaining a Red Colouring Matter from Ox Gallstones.*—The ox gallstones are powdered and sifted through cambric. Much dust is produced during this operation, and covers the operator with a pertinaciously adhering yellow colour. It is necessary to exclude the dust from the air-passages by cloths wrapped round face and neck, as it easily causes a furred tongue and fever when inhaled and swallowed. The powder is next stirred up with hot water in the same manner in which cooks are in the habit of mixing dough, to prevent the formation of lumps of dry dust. When every portion is well moistened a large quantity of hot water is added while the mixture is well stirred; it is then allowed to stand at rest for several days. The fluid is then separated from the deposit by decantation. If the operator is not desirous of examining the water extract of ox gallstones it is not worth while to filter this fluid, which remains turbid on account of a small quantity of suspended colouring matter. The powder is washed by the repeated addition and subsequent decantation of water, until at last it is placed upon a filter and washed pure by percolation. The paste is then transferred into a flask, a large quantity of strong alcohol added, and the mixture is digested and boiled in a water-bath. The alcohol extracts a free biliary acid, a lime salt of an organic fatty acid, and, rarely, a little cholesterin. The powder is exhausted with alcohol, lastly, on the filter placed in a hot funnel, until the alcohol is only faintly yellow, and on evaporation leaves only a little colouring but no resinous matter.

The exhausted powder is now treated with cold dilute hydrochloric acid, which causes the evolution of carbonic acid and sulphuretted hydrogen. It is better to allow the acid to act upon the powder for some days at the ordinary temperature, than to increase its dissolving power in time by the employment of heat. The powder is washed by decantation, lastly, on the filter, and treated a second time with alcohol. It now extracts but little biliary acid, but a brown colouring matter, which has evidently been set free by the acid (mainly bilirubin), which is slightly soluble in alcohol. After complete exhaustion with alcohol the residue may yet be treated with ether, but if the former operations have been completely performed this agent will extract little or nothing besides a little bilirubin.

After drying the powder is of a fine reddish-yellow colour, which speedily becomes red or brownish-red when exposed to the light. It is now boiled with pure chloroform, free from water and hydrochloric acid, care being taken to condense and cause to flow back into the flask the evaporated chloroform. The chloroform solution may now be filtered off, and the residue from the filter returned into the flask, to be anew boiled with chloroform. Large quantities of chloroform are of necessity lost during this operation. The powder may also be placed in a so-called exhauster, but then the advantage of being able to boil the chloroform with the powder is lost.

The following mode of operating is the most advantageous:—The solution obtained by boiling is allowed to stand at rest with the powder in the closed flask for 24 hours. After that time all powder has collected on the top of the fluid; the clear dark red solution fills the bottom of the flask. This solution is now drawn off by means of a syphon fixed in a cork, which closes a second flask, and which is partly emptied of air by suction with the mouth through a second tube piercing the cork, after the free end of the syphon has been immersed to the bottom of the flask containing the powder and solution. The fluid thus drawn off is obtained perfectly clear by filtration through paper. From the red solution most of the chloroform is distilled off in the water-bath. The residue is placed on a filter, washed with chloroform, then with absolute alcohol until the latter is nearly colourless, and the residue on the filter purely red, without any

admixture of green. The colouring matter thus obtained is red, and of exactly the same appearance as the red oxyde of mereury obtained by heating the nitrate. Absolute alcohol and ether extract from it no heterogeneous substance, and only little of the colouring matter itself. But it is well to purify it once more by solution in chloroform, and preeipitation of the concentrated solution by absolute alcohol.

After the chloroform has repeatedly acted upon the gallstone powder the amount of colouring matter extracted by each operation becomes exceedingly small. It is then advisable to treat the dried powder again with dilute hydroehloric acid, to wash it with water and spirit, and after drying to resume extraction with chloroform; or the dried powder may be treated with an alcoholic solution of caustic potash. The colouring matter dissolves with a dark reddish-brown colour, and can easily be separated from the sediment of impurities by filtration. The filtrate after acidulation with hydroehloric acid deposits the red colouring matter in flakes, which are quickly collected on a filter, and then boiled in absolute alcohol or shaken with chloroform. After cooling the preeipitate is collected on a filter, and washed with absolute alcohol. It is a yellowish powder, which owing to its finely subdivided state quickly becomes green on the surface by oxydation. In that case it must be again treated with absolute alcohol. The chloroform washings, which are dark green, should after distillation of some chloroform be preeipitated with absolute alcohol, to obtain the rest of bilirubin. From the various mother liquors much green colouring matter may be obtained, particularly from the first alcoholic acid solution, by addition of water.

*Purification of bilirubin with alcohol and soda. (a) First experiment.*—The total quantity used was 16 grammes. This was treated with alcohol of 85 per cent. containing 4 grammes of pure sodic hydrate prepared directly from the metal.

It was mostly soluble and was extracted with three successive portions, *vide* 1st, 2nd, and 3rd extracts. (The residue consisted of a black matter containing a few red particles, and was examined as follows: The substance was powdered with chloroform and heated twice to boiling, this extracted a small amount of yellow colouring matter, which was probably a trace of bili-



rubin, leaving the greater portion as an insoluble black powder which when dry weighed 0.9 gm.)

*The first and second extract* were thrown together and precipitated by hydrochloric acid in the least possible excess, filtered and washed, taking care that all chlorine should be eliminated, then washed with absolute alcohol, then with ether, lastly collected and dried *in vacuo*; in this manner the bilirubin was obtained as a hard, brittle substance, possessing considerable lustre, which when powdered became highly electric; the total quantity thus obtained weighed 12 grammes. This was now extracted by large volumes of chloroform and boiled until the whole was dissolved except a slight amount of black substance which was disregarded. The chloroform extracts were then concentrated to about half their bulk, and on cooling deposited 4.4 grms. of beautifully red bilirubin. On still further concentrating the mother liquor deposits to the extent of 3.2 grms. took place; there was also a ring which adhered to the side of the flask weighing 0.6 grms. The ultimate mother liquor evaporated and precipitated by absolute alcohol gave 2.4 grms., so that the several crops amounted to 10.6 grms.

*The third extract* was treated in the same manner as extracts N<sup>r</sup>. 1 and 2, but the product was much darker in colour. The insoluble residue weighed 0.1 gm.; the total chloroform extract weighed 0.3 gm.

(b) *Second experiment with absolute alcohol and soda.* Absolute alcohol containing a solution of pure caustic soda was placed on 8.7 grms. of bilirubin. After filtering, the filtrate was precipitated with hydrochloric acid, which produced the red precipitate of bilirubin; this was filtered and washed in the usual manner; the filtrate had a brilliant green colour. The red bilirubin was found to be slightly soluble on washing with alcohol; its solubility therein was therefore determined and found to be 0.0235 in 100 c.c. equal to 0.235 gm. in one litre.

The precipitated bilirubin which had become greenish-black during drying was finely powdered, giving a dark red powder. It was now treated with about two litres of chloroform and allowed to stand, and the solubility effected in the cold estimated as 1.72 grms. per litre; it was again treated with successive portions of hot chloroform, which on cooling deposited bilirubin

in the form of a vermilion powder floating on the top of the chloroform. The quantities obtained thus were 3 grms. and 2.1 grms., also 1.3 grms. on precipitation with alcohol, giving a total of 6.4 grms.

*Biliphæin and bilirubin.*—Before the discovery of the use of chloroform as a solvent of the colouring matter, only brown modifications of this substance were known and obtainable by the several processes then in use, particularly that employed by Heintz. These brown matters were termed biliphæin or cholophæin, according to the more or less puristic tendencies of authors. When however the red colouring matter was obtained by means of chloroform, it was generally assumed that the brown colour of former preparations had been a sign of impurity. The red colouring matter accordingly went by the name of cholerythrin or bilirubin, and was believed to be the only form of pure colouring matter of bile.

During many operations for the isolation of pure colouring matter of bile I regularly obtained two modifications, one being reddish-brown, the other of a purely red colour, like nitric oxyde of mercury. The brownish-red products were kept apart for further purification. The purified product showed a darker, somewhat purple-brown colour, but in all other respects behaved like the purest bilirubin. On microscopic examination it was found to consist of large crystalline particles, mixed with many perfect crystals. The bilirubin on the other hand consisted almost entirely of minute amorphous granules; only when it had been precipitated by alcohol it showed minute yellow rhombic prisms. When the mixture of chloroform solution and alcohol, from which the first or immediate precipitate of bilirubin had been removed by filtration, was allowed to stand and mixed gradually with some more alcohol, it deposited another quantity of colouring matter, partly red, partly brown, the former amorphous, the latter in large crystals united to clusters. The brown crystalline part could be separated by lœvigation with alcohol, in which it settled quicker than the red matter.

The crystals thus isolated had a dark reddish-brown colour; their surfaces reflected the light with a purple and steel-blue glitter. They were opaque under the microscope; thin scales appeared reddish or red; the very thinnest crystals, of which

there were few, transmitted the light with a yellow colour. The crystals were mostly from one-tenth to one-eighth of an inch in length, of from one-twentieth to one-thirtieth of an inch in width, and their third diameter was probably from one-hundredth of an inch to immeasurable thinness. By their form they belonged to the rhombic system, being prisms in some crystallisations simple and with obtuse nearly right angles, in others with sharp angles on the one, obtuse angles on the next edge, and planes from secondary prisms cutting off the sharp angles of the primary ones.

The most minute crystals of bilirubin also showed the same shape, and were of a yellow colour. The red modification could by cautious crystallisation always in part be transformed into the brown. Without special elementary analysis of the two modifications it may be considered as proved that this colouring matter appears in two modifications, a crystallised or crystalline one of purple-brown colour, cholophæin or biliphæin, and an amorphous one of a bright red colour, bilirubin.

This observation will explain the discrepancies concerning this substance which have existed in the writings of many authors. It disproves the statement of Städeler that the pure colouring matter of bile was red only, and that when a product of this kind was not red but brown and crystallised it was impure. The pigment can however not always be obtained crystallised at will, as is shown by the following experiment.

About 10 c.c. of concentrated chloroform solution of bilirubin was treated with an equal volume of absolute alcohol in such a way that the alcohol rested as a separate layer on the top. This mixture was now allowed to evaporate spontaneously, and was found to give no crystals, but only a granular deposit even when evaporated nearly to dryness.

In the following I shall consider cholophæin and bilirubin as identical chemical substances: but whenever the one or the other name is used in the description of a process, the reader will understand that the modification indicated by the name has been taken for the experiment.

The red, nearly orange-coloured amorphous colouring matter when exposed to light, while all moisture is carefully excluded, gradually assumes a brown colour on its surface. In the

interior the powder remains unchanged for a long time. When boiled with water for some time it assumes the same brown colour. In water it is quite insoluble, slightly soluble in boiling absolute alcohol, with a yellow colour; on filtration through paper the colouring matter of the first portions of alcoholic solution remains adhering to the paper fibres, and the alcohol passes nearly white.

In ether it is very little soluble, somewhat easier in sulphide of carbon and benzol. The best solvent is chloroform, of which 200 parts dissolve about one part of colouring matter.

10 c.c. left on distillation 0.074 grm., equal to 0.74 grm. per 100 c.c., and as the ten c.c. of solution weighed 14.859 grms. a hundred grm. contained 0.498 grms. of bilirubin.

The rays of the sun discolour the chloroform solution; the addition of hydrochloric acid produces a precipitate in it.

*Elementary Composition of Bilirubin.*—Bilirubin when prepared as above and dried *in vacuo* lost yet some water and then was constant. By many varied elementary analyses, the details of which I have communicated in *Journ. f. pract. Chem.* 104 (1868) 1, its composition was ascertained to be  $C_9H_9NO_2$ .

Atoms	Atomic weights	In 100
9 C . .	108	66.26
9 H . .	9	5.52
N . .	14	8.59
2 O . .	32	19.63
	<hr/> 163	<hr/> 100.00

*Compounds of Cholophæin. Neutral Monohydrated Cholophæinate of Silver.*—A neutral solution of cholophæin in ammonia, prepared by digesting a dilute ammonia solution in water with excess of cholophæin, was precipitated with nitrate of silver. The precipitate was of a reddish-brown colour, and after washing with water was dried in the vacuum over sulphuric acid, light being completely excluded.

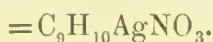
The mean of several determinations gave 37.39 per cent. of silver.

Taking into consideration that the analyses of cholophæin or bilirubin led to the empirical formula  $C_9H_9NO_2$ , there cannot be any doubt but that the quantity of silver found in the



cholophæinate is exactly that required by a neutral monohydrated compound of the formula  $C_9H_{10}AgNO_3$ . However anomalous may be a silver salt containing an atom of water, it is now certain that the composition and molecule of cholophæin is expressed by the formula  $C_9H_9NO_2$ .

Theory of cholophæinate of silver dried *in vacuo*



Symbols		Atomic weights	Percentages
$C_9$	.	108	37·50
$H_{10}$	.	10	3·47
$Ag$	.	108	37·50
$N$	.	14	4·86
$O_3$	.	48	16·67
		288	100·00

*Basic Anhydrous Cholophæinate of Silver.*—An ammoniacal solution of cholophæin (which cholophæin had been repeatedly purified by solution in chloroform and in caustic potash and alcohol) was treated with nitrate of silver. No precipitate appearing, an excess of silver was added, and then some nitric acid to neutralise as nearly as possible the excess of ammonia. A precipitate now formed, the solution became perfectly colourless, and the precipitate was washed with water. It was dried *in vacuo* at  $130^\circ C$ .

It is thus shown that cholophæinate of silver is soluble in free ammonia, and that when the ammoniacal solution, in the presence of an excess of silver solution, is neutralised down to a proper degree, the basic salt falls down. Its theory is derived from the data concerning free cholophæin, the neutral silver salt, and from direct analysis its formula is  $C_9H_7Ag_2NO_2$ . In this compound two atoms of hydrogen are replaced by two of silver. An analogous lead compound in which the two atoms of hydrogen are replaced by one didynamic atom of lead will be described lower down. Its formula is  $C_9H_7PbNO_2$ , and its production strengthens the presumptions in favour of the basic silver compound.

Theory of basic cholophæinate of silver dried *in vacuo*



Symbols	Atomic weights	Percentages
C <sub>9</sub> . .	108	28·64
H <sub>7</sub> . .	7	1·85
Ag <sub>2</sub> . .	216	57·29
N . .	14	
O <sub>2</sub> . .	32	
	<hr/> 377	

*Neutral Cholophæinate of Baryum.*—An ammoniacal solution of cholophæin with excess of ammonia was precipitated by chloride of baryum. The dark greenish-brown precipitate was washed until the filtrates were free from baryum; the filtrates were all somewhat green-coloured, and on standing deposited green flakes. The compound was dried at 100°, and formed a dark brown powder.

The analyses yielded data corresponding to the requirements of a baryum compound, which is strictly analogous to the neutral silver salt, containing a didynamic atom of baryum, which solders together two molecules of cholophæin by replacing one atom of hydrogen in each of them; two molecules of water are superadded.

Theory of baryum cholophæinate =  $C_{18}H_{20}BaN_2O_6$ .

Symbols	Atomic weights	Percentages
C <sub>18</sub> . .	216	43·46
H <sub>20</sub> . .	20	4·02
Ba . .	137	27·56
N <sub>2</sub> . .	28	„
O <sub>6</sub> . .	96	„
	<hr/> 497	

*Half-acid Cholophæinate or Sesqui-cholophæinate of Baryum.*—This salt, prepared by precipitation from a neutral ammoniacal solution by BaCl<sub>2</sub>, washing with water, treating and boiling with alcohol until the alcohol on filtration and washing passed clear and colourless, was dried in the steam closet. It remained of a brownish-red colour, but after being powdered it became of a darker brown colour on the surface during drying. Dried at 100° until constant, and then at 110° C., it yielded on analysis data which showed that in this

cholophæinate one atom of baryum is in combination with three molecules of cholophæin. If to the dihydrated neutral cholophæinate of baryum described above we add one molecule of cholophæin, thus—

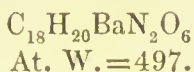
1 cholophæinate of baryum	$C_{18}H_{20}BaN_2O_6$	= 497	At. w.
1 cholophæin	$C_9H_9N_1O_2$	= 163	„
We obtain	$C_{27}H_{29}BaN_3O_8$	= 660	„

To the theory of this compound the above analyses exactly correspond, as is evident from the following comparison:—

Symbols	Atomic weights	Percentages	Found mean
$C_{27}$	324	49.09	50.63
$H_{29}$	29	4.39	4.37
Ba	137	20.75	20.66
$N_3$	42		
$O_8$	128		
	660		

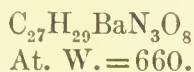
By the following comparison the differences in the composition of the neutral and the half-acid cholophæinate of baryum will be exhibited in a striking manner.

NEUTRAL CHOLOPHÆINATE OF  
BARYUM.



	Theory	Found
C . .	43.46	44.58
H . .	4.02	3.98
Ba . .	27.56	27.55

HALF-ACID CHOLOPHÆINATE OF  
BARYUM.



	Theory	Found
C . .	49.09	50.63
H . .	4.39	4.37
Ba . .	20.75	20.66

*Neutral Cholophæinate of Calcium.*—The excess of cholophæin, which had served to make the neutral-ammoniacal solution used for the production of cholophæinate of silver, was dissolved in a slight excess of ammonia and precipitated by chloride of calcium. The precipitate was red. Dried at 100° it yielded on analysis data which led to the formula of a neutral cholophæinate of calcium  $C_{18}H_{20}CaN_2O_6$ , a salt in every respect

analogous to the neutral baryum compound above described. We must assume in it the existence of two molecules of water, which are not expelled even at the temperature of  $100^{\circ}$ . The following comparison of theory with the analytical data will make the correctness of these conclusions more conspicuous:—

Symbols		Atomic weights	Percentages	Found mean
$C_{18}$	. .	216	54.00	53.86
$H_{20}$	. .	20	5.	4.90
Ca	. .	40	10.	10.17
$N_2$	. .	28		
$O_6$	. .	96		
		<hr/> 400		

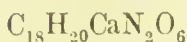
*Half-acid Cholophæinate or Sesqui-cholophæinate of Calcium.*—I had first of all cholophæinates prepared the baryum salt, which was found to be a sesquisalt. The neutral calcium salt was next obtained, and indicated the existence of two classes of salts, neutral and half-acid. The correctness of this conclusion was put to the test by the endeavour to obtain the neutral baryum salt, and the experiment proved successful. It was next necessary to obtain the half-acid cholophæinate of calcium. A neutral solution of bilirubin was therefore precipitated by chloride of calcium washed and dried.

The compound was found to be analogous to the half-acid baryum compound already described, and has the formula  $C_{27}H_{29}CaN_3O_8$ , and the following theory:—

Theory			
of atoms		of percentages	
$C_{27}$	. .	324	57.54
$H_{29}$	. .	29	5.15
Ca	. .	40	7.1
$N_3$	. .	42	
$O_8$	. .	128	
		<hr/> 563	

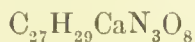
The following comparison exhibits the differences in the composition of the neutral and the half-acid cholophæinate of calcium.



NEUTRAL CHOLOPHÆINATE OF  
CALCIUM.

At. W. = 400.

	Theory	Found
C . . .	54·	53·86
H . . .	5·	4·9
Ca . . .	10·	10·17

HALF-ACID CHOLOPHÆINATE OF  
CALCIUM.

At. W. = 563.

	Theory	Found
C . . .	57·54	—
H . . .	5·15	5·74
Ca . . .	7·1	6·91

In his researches on the colouring matter of human gall-stones, Städeler produced a combination of bilirubin with calcium, which on analysis yielded him 9·1 per cent. of calcium oxyde. Assuming this compound to be the normal neutral salt, he allowed the atomic weight of bilirubin to be thereby determined, and rejecting his former analyses of crystallised cholophæin as given in Frerichs' *Treatise on Diseases of the Liver*, he abandoned the empirical formula  $\text{C}_{18}\text{H}_9\text{NO}_4$ , and substituted  $\text{C}_{32}\text{H}_{18}\text{N}_2\text{O}_6$  as the formula of bilirubin, and  $\text{C}_{32}\text{H}_{17}\text{CaN}_2\text{O}_6$  as that of bilirubate of calcium. (The foregoing three formulæ are given in the old notation.) As this formula is supported by only one, and that a very unsatisfactory calcium determination, and as all the analyses of Städeler concerning bilirubin or cholophæin can be brought into harmony with my results, I can have no hesitation in considering his formulæ, both of bilirubin and bilirubate of calcium, as erroneous. The bilirubate analysed by Städeler was evidently the sesquisalt.

Theory of  $\text{C}_{27}\text{H}_{29}\text{CaN}_3\text{O}_8$  re-  
quires

CaO or Ca

9·94 per cent. 7·1 per cent.

Städeler found

CaO or Ca

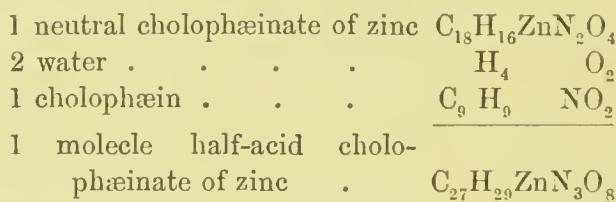
9·1 per cent. 6·5 per cent.

With Städeler's formula of bilirubin fall the formulæ of all other substances described by him under the names of biliverdin, biliprasin, bilifuscin, and bilihumin.

It remains to point out in this place the circumstances under which cholophæin will form half-acid, neutral, or basic salts. A watery solution of ammonia which is saturated with

cholophæin by digestion with an insoluble excess yields on the addition of neutral salts of metals the sesquisalt. Possibly the ammonia salt is itself a sesquisalt while dissolved in water. From somewhat alkaline solutions the basic salts of silver and lead are precipitated by the opportune addition of an excess of metallic salt. But neutral salts of earths added to the solutions containing excess of ammonia precipitate the neutral compounds with earths.

*Cholophæinate of Zinc.*—A neutral ammoniacal solution of cholophæin was precipitated with sulphate of zinc. The reddish brown precipitate was easily washed, and quite insoluble in water. Dried at 100° C. it was subjected to analysis. From the amount of zinc found it is evident that this compound is constructed upon the plan of the half-acid salts of calcium and baryum. Thus—



Theory of  $C_{27}H_{29}ZnN_3O_8$

Atoms	Sums of Atomic weights	Percentages
$C_{27}$ . . . . .	324	—
$H_{29}$ . . . . .	29	—
Zn . . . . .	65	11·05
$N_3$ . . . . .	42	
$O_8$ . . . . .	128	
	588	

*Neutral Cholophæinate of Lead.*—Precipitated from a neutral solution by acetate of lead. Dried at 100°. Figures were obtained which keep in proximity of those required by a neutral dihydrated cholophæinate  $C_{18}H_{20}PbN_2O_6$ . At. W.=567, Pb=36·50 per cent.

The half-acid cholophæinate of lead  $C_{27}H_{29}PbN_3O_8=730$  requires 28·21 per cent. of Pb.

*Basic Cholophæinate of Lead.*—The compound was precipitated by acetate of lead in great excess from a neutral ammoniacal solution of cholophæin, which although it did not dissolve any more cholophæin, smelled of ammonia, and had an alkaline reaction. Dried at 100° C.

This compound may be explained as being a basic cholophæinate, or cholophæin, in which two atoms of hydrogen are replaced by one didynamic atom of lead.

Atoms	At. weights	Percentages	Found mean
C <sub>9</sub> . .	108	—	—
H <sub>7</sub> . .	7	—	—
Pb . .	207	56·25	58·1
N . .	14		
O <sub>2</sub> . .	32		
	<hr/> 368		

This compound corresponds to the basic-cholophæinate of silver, or binargentic cholophæin, described above, C<sub>9</sub>H<sub>7</sub>Ag<sub>2</sub>NO<sub>2</sub>.

*Neutral Cholophæinate of Copper.*—This salt was precipitated by sulphate of copper from a neutral solution.

*Basic Cholophæinate of Copper.*—This salt was obtained by precipitating an ammoniacal solution with excess of acetate of copper.

The only probable formula for this compound is that of a cholophæinate containing 2 cholophæin and 3 copper.

Atoms	Atomic weights	Percentages	Found
C <sub>18</sub> . .	216	—	—
H <sub>12</sub> . .	12	—	—
Cu <sub>3</sub> . .	190·5	37·31	38·70
N <sub>2</sub> . .	28		
O <sub>4</sub> . .	64		
	<hr/> 510·5		

*Action of oxygen upon bilirubin in alkaline solution. Cholochlorin or biliverdin.*—When bilirubin is dissolved in caustic potash and exposed to the air, it becomes gradually green. This change can be accelerated by warming the solution and

passing a current of air through it. When in thin layers it is purely green, without any admixture of red or yellow, the reaction is complete. On addition of hydrochloric acid a green matter is precipitated in flakes, which is first washed by decantation, and lastlyedulcorated on the filter. This is biliverdin.

*Chemical Properties.*—Biliverdin is easily soluble in alcohol with a splendid green colour, particularly while in the wet state; after drying it is soluble with great difficulty only; it is more soluble in hot than in cold alcohol. It is soluble in hydrochloric acid, forming a green solution, in which platinum tetrachloride and mercury dichloride produce amorphous green precipitates. A quantity of alcoholic solution boiled down with tincture of iodine, and the residue shaken with dilute caustic potash, left a greenish-black resin, which was not soluble in sulphuric acid except under evolution of sulphurous acid, indicating decomposition. By reducing agents the biliverdin cannot be retransformed into cholophæin. Dissolved in caustic potash and treated with hydrothion it assumes a greenish-brown colour, but the original red is not produced. When metallic zinc is added to a solution of biliverdin in hydrochloric acid, the green colour disappears and gives way to a brownish-red one, but the produce is insoluble in chloroform. An alkaline solution treated with sodium-amalgam changes its colour to a reddish-brown, and a small quantity of a brown matter is deposited in flakes. But the reddish-brown matter on exposure to air does not become green again. Hydrochloric acid precipitates a brown flaky matter from the alkaline solution.

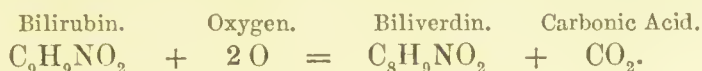
When chlorine gas is passed into water containing biliverdin in suspension, dark yellow flakes are obtained, which while insoluble in water and ether dissolve easily in alcohol. When a few bubbles of chlorine gas are conducted into an alcoholic solution of biliverdin the dark-green fluid is instantaneously discoloured. After evaporation of the alcohol, yellowish white flakes remain, which by a gentle heat fuse to a reddish-yellow mass. They contain chlorine, and are a mixture of at least three compounds, which can be separated by chloroform.

Biliverdin when repeatedly purified and subjected to varied analyses gave data which led to the formula  $C_8H_5NO_2$ .



Theory				Mean found
of atoms		in 100		
8 C	. . 96	63·57		62·94
9 H	. . 9	5·96		6·13
N	. . 14	9·27		9·34
2 O	. . 32	21·20		22·10
	<hr/> 151	<hr/> 100·00		<hr/> 100·00

This shows that biliverdin arises from bilirubin by the addition of 2 oxygen, and the subsequent subtraction of 1 carbonic acid.



On boiling an alcoholic solution of biliverdin with caustic ammonia and nitrate of silver, a reduction of the dissolved silver oxyde is observed. The solution remains green, but on addition of an acid, such as nitric, hydrochloric, or sulphuric, assumes a splendid purple colour. The new body produced is a product of oxydation and termed bilipurpin.

The alcoholic solution of biliverdin is not altered by boiling with red oxyde of mercury. Lead peroxyde immediately changes its colour to brown, and precipitates a portion of the dissolved matter. The same change of colour without any accompanying precipitation is produced by peroxyde in the presence of ammonia. A mixture of peroxyde of lead and sulphuric acid changes the colour of the alcoholic solution to a light yellow, and evolves aldehyde and acetic ether from the alcohol.

Biliverdin dissolves easily in caustic alkalies, and is not precipitated from their solution by any metallic salt. A saturated solution in alcohol gives a precipitate with baryta water. When this reagent is added until a sample filtrate is only pale green, and boiled, all biliverdin is in combination. It must be filtered quickly, air being excluded, and washed with strong alcohol. It cannot be washed with water, as the purer it becomes the more soluble it is, and if washing with water were indefinitely continued it would be dissolved entirely. The analyses of this salt led to the conclusion that it contains three molecules of biliverdin and one didynamic atom of baryum, and has the

formula  $C_{24}H_{27}BaN_3O_7$ , or  $C_{24}H_{25}BaN_3O_6 + H_2O$ . This is half-acid biliverdate with one molecule of water, according to the following theory:—

1 mol. neutral biliverdate	$C_{16}H_{16}BaN_2O_4 = 437$
1 mol. biliverdine . . .	$C_8H_9N_3O_2 = 151$
1 mol. water . . .	$H_2O = 18$
<hr/>	
1 mol. of half-acid biliverdate, $C_{24}H_{27}BaN_3O_7$	$= 606$

Atoms	At. weights	Percentages	Mean found
24 C . .	288	47.52	48.79
27 H . .	27	4.45	4.38
Ba . .	137	22.60	22.41
3 N . .	42		
7 O . .	112		
<hr/>		606	

If the one molecule of water be omitted from the calculation, the theory of carbon corresponds better with the experience, but that of the baryum less well.

Atoms	At. weights	Theory of percentages	Mean found
$C_{24}$ . .	288	48.97	48.79
$H_{25}$ . .	25	4.25	4.38
Ba . .	137	23.29	22.41
$N_3$ . .	42	7.14	
$O_6$ . .	96		
<hr/>		588	

Lime water yields a precipitate in an alcoholic solution of biliverdin, which does not, however, appear to be a compound in atomic proportions but a mixture of such a one with excess of free biliverdin.

Lead acetate produces a precipitate in the alcoholic solution of biliverdin, which is more soluble in hot than cold alcohol. Basic lead acetate produces a voluminous precipitate, which is so complete that the fluid has the merest green tinge. Acetate of mercury also produces a precipitate more soluble in hot than cold alcohol. Acetate of copper produces a brownish green precipitate, and the filtrate is nearly colourless. The precipi-

tates of lead, mercury, and copper can be washed with water without dissolving. Freshly precipitated silver-oxyde does not combine with biliverdin without change, but produces oxydation. The derivate is yellow, and soluble in water and alcohol.

In an experiment instituted to measure the quantity of oxygen necessary for the transformation of a given quantity of bilirubin into biliverdin, by means of the deoxydation of an alkaline copper solution, several copper compounds were produced, of which two contained the copper in such a form that it could not be removed by carbonated soda, but followed the product into the alcoholic solution. One of these products had a peculiar spectrum, and exhibited an absorption band in blue when viewed by the aid of Drummond's light.

*Action of Bromine upon Biliverdin.*—In the following experiments a quantity of biliverdin was used, which had been prepared from bilirubin by Heintz's process, and on analysis had yielded the formula  $C_8H_9NO_2$ .

1. *Monobrominated biliverdin.*—A quantity of finely powdered biliverdin, which when quite dry at  $100^\circ$  weighed 0.8064 gm., was treated in a Liebig's drying apparatus with dry bromine vapour mixed with dry air. It absorbed the bromine and became perfectly black. When the action of bromine had been allowed to complete itself during many hours, at the ordinary temperature, the excess of bromine was displaced by dry air. The product now weighed 2.3684 grms., or almost threefold the weight of the original biliverdin. The apparatus was now heated to  $100^\circ$  and dry air passed over the product for many hours. A little bromine and much hydrobromic acid escaped, and after the passing of more than two hundred litres of air (measured by the displacement of the water in the aspirator) the apparatus became of constant weight, and the substance lost mere vestiges of HBr. It now weighed 1.22236 gm. The equation



requires that 151 parts biliverdin should become 230 parts of brominated substitution-product, and therefore the 0.8064 biliverdin should have increased to 1.2282, equal to an addition of 0.4218 gm. of bromine. This hypothesis is therefore very nearly satisfied by the experiment.

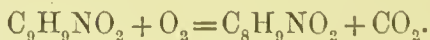
The new product is a perfectly black powder, insoluble in ether, very little soluble in alcohol; soluble in oil of vitriol, precipitated from this by dilution with water; the solution has a feeble purplish tint; soluble in caustic soda, precipitated from this in brown flakes by acetic acid. From the aspect of the reaction it is probable that the substance cannot be dissolved in either sulphuric acid or soda without change.

*Analysis.*—*a.* 0.0282 grm. burned with copper oxyde in *vacuo* yielded a mixture of gas which after the necessary corrections amounted to 23.78 c.c. Of this 22.4 c.c. were CO<sub>2</sub>, and 1.38 were N. This is equal to 42.58 per cent. C, and 6.12 per cent. N. The relation of C : N is therefore = 8.1 : 1.0.

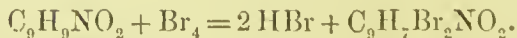
*b.* 0.1920 grm. ignited in glass tube with soda and nitre, &c., gave 0.1613 AgBr, equal to 35.72 per cent. Br.

*c.* A combustion with lead chromate in a very long tube yielded quantities which showed that the quotient of carbon by its atomic weight was to that of hydrogen as 3.22 to 3.179, or, in atoms, very nearly 8 : 8. These analyses, therefore, confirm the presumption derivable from the synthetical genesis of the product, namely that it is C<sub>8</sub>H<sub>8</sub>BrNO<sub>2</sub>. Theory requires 41.73 per cent. C; 3.47 per cent. H; 34.73 per cent. Br; 6.08 per cent. N; leaving for O 13.99 per cent.

This reaction, therefore, confirms the formula which in consequence of my researches, first communicated in the *Tenth Report of the Medical Officer of the Privy Council*, 1867, p. 240 to 251; also *Jour. pr. Chem.*, 104 (1868), 4 *et seq.*, and *Proc. Roy. Soc.* xvi. 217, I have attributed to biliverdin, namely C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>. Incidentally the reaction by which bilirubin is transformed into biliverdin is also confirmed, and some additional light is thrown on the process;



Bilirubin (as I have shown in *Chem. Soc. J.*, May 1875, p. 389, and more fully in a chapter below) when treated with dry bromine vapour, yields up two atoms of hydrogen, and assumes two atoms of Br in their place:



It is therefore clear that bilirubin, when passing into biliver-



din, not only loses an atom of carbon, but also undergoes a change regarding the manner in which one of its atoms of hydrogen is bound, so that this hydrogen-atom, though capable of being replaced in bilirubin, is no longer replaceable by Br in biliverdin.

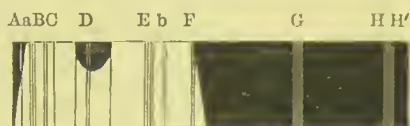
2. *Hydrobiliverdin*.—A quantity of biliverdin was dissolved in caustic soda and water, and some sodium-amalgam added. The mixture was repeatedly agitated. On the third day the solution, at first greenish, was brownish-red. Hydrochloric acid now gave a brown deposit which was collected on a filter andedulcorated with water. The filtrates were coloured reddish, and seemed to contain a side product, soluble in dilute HCl. The precipitate was treated with alcohol, and dissolved to a great extent, but a portion remained insoluble in even boiling alcohol, and ultimately formed a black powder. The alcoholic solution contained, however, the bulk of the new product.

*Spectral Phenomena of the Alcoholic Solution of Hydrobiliverdin*.—The concentrated dark-brown solution, with Drummond's light, transmits red only. On greater dilution, all red, yellow, and some green rays pass; green is shaded. Again more diluted, a separate feeble absorption-band appears at the junction of green and blue, overlying the line F equilaterally. This spectrum is, therefore, not identical with that of hydrobilirubin (see diagrams of both spectra below, p. 295.), in which the absorption-band fills the space between the lines E and F, overlapping them both, and is of unsymmetrical intensity, its greatest intensity being about one-third nearer to E than F. This experiment thus leads to a presumption that hydrobiliverdin and hydrobilirubin, though similar in external appearance and some properties, are not, as has been alleged, identical.

The alcoholic solution of hydrobiliverdin is precipitated with water. Ammoniacal solution of zinc chloride added to this dissolves all, and the solution fluoresces feebly greenish-brown in sunlight only; the fluorescence appears to be homochromatic.

*Product of Bilirubin and fuming Sulphuric Acid*.—This substance when examined presented the following characters: (a) Slightly soluble in water. (b) Insoluble in ether and benzol. (c) Slightly soluble in alcohol, giving a blue-green solution and a specific spectrum. (d) Soluble in ammonia to a

dark green almost black solution, remarkable for its powerful absorption of light, which when diluted, presented the following spectrum: 1 c.c. thick, not transparent; further diluted, red and blue appear, but very dark indeed; again diluted, transparent green with feeble band.



*Bilirubin changed by fuming Sulphuric Acid, dissolved in Ammonia.*

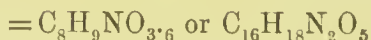
Alcoholic solution: All colours were very much obscured. Red nearly out.

(a) 0.396 gave 0.7800 of carbonic anhydride equal to 53.718 per cent. of carbon, and 0.186 of water equal to 5.218 per cent. of hydrogen.

(b) 0.439 gave 30.5 c.c. gas at 20° C, and 767 m.m. Potash column equal to 16.7 m.m. mercury; equal to 27.4 c.c. Nitrogen normal, or to 7.80 per cent. of N.

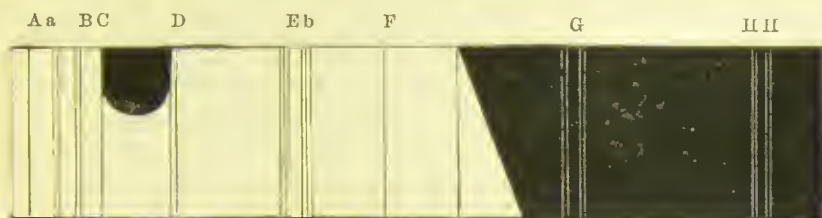
	Per cent.	÷ At. wts.	÷ N = 1
Carbon .	53.718	4.4765	8.03
Hydrogen .	6.218	5.2180	9.3
Nitrogen .	7.800	.5571	1.0
Oxygen .	33.264	2.0165	3.6

100.000



*Sulphate of Sulpho-cholocyanin.*—Cholophæin was treated with fifteen times its weight of sulphuric acid hydrate. After standing six hours it had assumed a reddish-green black colour. Diluted with sulphuric acid it was almost impenetrable to light. More diluted red, then a dark band, then some light, but no green, blue or violet was seen. More diluted there appeared red, then band green and blue. This band in the concentrated acid solution is broader in both directions than the band of sulphate of cholocyanin in dilute watery solution prepared by fuming sulphuric acid. The line near D was difficult to read, and therefore somewhat doubtful. A second reading of a dilute

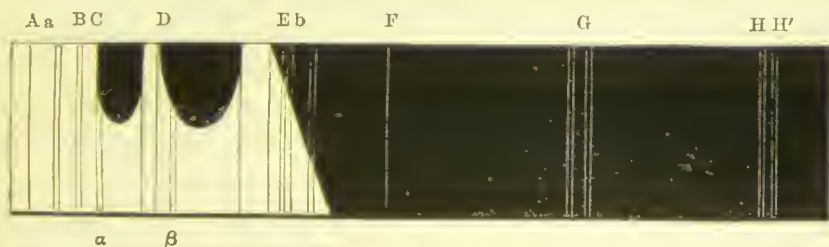
solution and a third observation of the spectrum of the concentrated solution between two glass plates, so that a very thin layer



*Diagram of Spectrum of Sulphate of Sulpho-Cholocyanin.*

only had to be traversed by the light, yielded data from which the diagram was constructed.

*Cholothallin.*—The concentrated solution just described on dilution with much water deposits a green precipitate, of which a portion is insoluble, another soluble in alcohol. The insoluble portion, cholothallin, has the composition  $C_9H_{11}NO_3$ , and is therefore hydrated cholophæin, an isomer of tyrosin. The soluble portion has in solution a red colour, and shows two remarkable bands of absorption, resembling somewhat the bands of blood, but being situated in different parts of the spectrum, i.e. more towards the red end than those of blood.



*Diagram of Spectrum of Soluble Cholothallin.*

The green ends in faintest bluish-black, and the spectrum altogether at b.

*Action of bromine and hydrobromic acid on bilirubin.*—1.0115 grm. of dry bilirubin were suspended in 1 litre of dry chloroform and to it was added a mixture of bromine and hydrobromic acid, prepared by treating potassic bromide with sulphuric acid, and made absolutely dry by being passed over pumice and sulphuric acid, until the hydrobromic acid was unabsorbed. The chloroform was distilled off in the water-bath ;

at first hydrobromic acid and bromine alone, and then bromine and chloroform were evolved; during the distillation the apparatus, which was perfectly closed, became a vacuum, all caoutchouc tubes collapsing. When the chloroform was low and the colour perfectly green, the distillation was stopped and water added; immediately all colouring matters were deposited in nearly black, greenish, violet, blue flakes, which on standing became hard.

These flakes while still moist were treated with ether as long as anything was extracted, giving a perfectly violet solution, the ether was now distilled off, and a green matter remained, showing that the violet body had been changed.

The flakes were not wholly soluble in ether, but left a green body which was easily soluble in alcohol and on evaporation left a green, very hygroscopic body.

A small quantity of matter was insoluble in ether or alcohol and had a dark green colour. Therefore the main product was the violet dibromobilirubin, which in the presence of water hydrobromic acid and ether changed into a green body.

The following remarks are to be made about this preparation:—

(1) Pumice and sulphuric acid were used, and as the pumice was afterwards found to contain hydrochloric acid, it must have contaminated the experiment, although but slightly. (2) Much sulphurous anhydride must have been evolved by the action of sulphuric acid on hydrobromic acid. Therefore there must have been three main actions.

(a) That by hydrobromic acid; (b) that by bromine; and (c) that by sulphurous anhydride, besides a slight action of the hydrochloric acid.

Each action will have to be accounted for separately in future experiments.

The residues from the extracts when weighed gave: ether extract, 0.8300; alcohol extract, 0.6530; sediment, 0.4995, giving a total of 2.083. The bromine was then determined in the residue from the ether extract; 0.2445 gave 0.0968 of argentic bromide, equal to 16.85 per cent. of bromine, and from a second portion of 0.3070, 0.1213 argentic bromide was obtained, equal to 16.808 per cent. of bromine.



*Bilirubin and hydrobromic acid.*<sup>1</sup>—1.016 grm. of dry bilirubin was suspended in a litre of pure dry chloroform, and pure hydrobromic acid added, and the mixture allowed to stand for 48 hours, when a green solution resulted. The chloroform was now distilled off; at first hydrobromic acid was evolved in large quantities, afterwards chloroform saturated with hydrobromic acid.

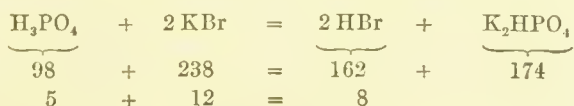
The residue was made perfectly dry by passing a current of dry air at 100° C. over it.

The product thus obtained weighed 1.3916 grm., and was green by transmitted light in thin layers, but in thick layers reflected a light that was violet and golden.

*Analyses.*—The substance was first dried in a Liebig's apparatus, and then at 100° C. in the air bath. (a) 0.1438 grm. gave argentic bromide 0.0666, equal to 19.68 per cent. of bromine. Theory required 20.56 per cent.; (b) 0.374 grm. gave 25 c.c. N at 14° C. and 769 m.m. B. Potash column, equal 10.2 m.m. Mercury; equal to 24.3 c.c. normal nitrogen, or 8.12 per cent. Theory, 7.19. (c) 0.3872 gave carbonic anhydride 0.7146, equal to 50.33 per cent. of carbon and 0.1830 water, equal to 5.25 per cent. of hydrogen.

	Per cent.	÷ At. wts.	÷ N = 2.	÷ Br = 1.
Carbon . .	50.33	4.194	14.4	17
Hydrogen . .	5.25	5.25	18.0	21
Nitrogen . .	8.12	0.580	2.00	2.3
Oxygen . .	16.62	1.038	3.4	4.2
Bromine . .	19.68	0.246	0.84	1

<sup>1</sup> The hydrobromic acid was prepared by the action of phosphoric acid on potassic bromide, and the resulting hydrobromic acid well dried according to



5 grms. glacial phosphoric acid, 12 grms. potassic bromide taken.

Protest must be entered here against a common practice of traders of selling in the shape of sticks obtained by fusion, a product, which, while sold at a high price as 'pure glacial phosphoric acid,' actually contains about one-third, or, at all events, large quantities of sodic phosphate. This salt is added by the makers to give to their product the deceptive appearance and brittleness. The practice amounts to a fraud in every case where the buyer is not informed of the admixture of the phosphate.

The theory of a bromobilirubide-bilirubin,  $C_9H_8NOBr + C_9H_9NO_2$  requires :—

Carbon . . .	53·06
Hydrogen . . .	4·66
Nitrogen . . .	6·87
Oxygen . . .	15·72
Bromine . . .	19·65

*Bilirubin and bromine.*—0·5570 grm. of dry bilirubin was saturated at the ordinary temperature with dry bromine vapour. The following phenomena were then observed : (1) No green colour at any period, but only brown-red, or chocolate. (2) The powder became warm. (3) No fusion at any time. (4) The bromine was quickly absorbed at the beginning of the experiment, and slowly towards the end. When the whole of the powder, which was from time to time shaken, was of a deep purple colour, and bromine passed unchanged, the action was considered complete; but the bromine was allowed to be in contact for 12 hours longer. The product was dark purple, nearly black, and weighed 1·1646 grm. This corresponded to an addition of a little more than four atoms of bromine,  $163 : 320 = 0·557 : 1·0934$ . The excess of bromine is therefore 0·0712. This was evolved on subsequent heating. More air passed would, perhaps, have removed it. Theory shows that the compound produced is



This is not a very stable compound; the combination of the hydrobromic acid being very loose, it is not evolved by passing dry air at an ordinary temperature, but is completely removed by a long-continued current at 100° C. On heating it shrinks, but does not fuse, and on cooling becomes again pulverulent.

The product is accounted for thus:—0·5570 grm. taken; after bromine equal to 1·1646;  $C_9H_9NO_2 = 163$ ;  $Br_2 = 160$ .

$$163 : 160 = 0·557 : 0·5467$$

Theory 1·0934; Found 1·1646

Dried at 100° C. 0·5312 bromine was found to have been

added, which leads to 165 as being the atomic weight of bilirubin.

*Hypothesis.*—Either the adhesion of the two molecules of hydrobromic acid to the dibromo-bilirubin is a peculiar kind of loose combination produced by a chemism or attraction of the dynamicities corresponding to the hydroxyles contained in the bilirubin :—

Or one molecule of hydrobromic acid is combined as hydrobromate simply, attached to the N pole of the new compound ; and the other is differently attached, viz. by the transparent dynamicities, or the excess of attraction, which the dynamicities occupied by the hydroxyl have yet for the hydrobromic acid. The combination is destroyed at 100° C, and hydrobromic acid goes away. The fact that both molecules of hydrobromic acid go away at 100° C., and apparently without much change of the residue, except in volume, makes it probable that both of them are bound in an equal manner.

*Ultimate Analysis of Dibromobilirubin.*—(a) 0.1066 grm. gave 0.1210 of argentic bromide, equal to 48.31 per cent. of bromine. Theory requires 49.84.

0.385 grm. gave carbonic anhydride 0.4750 equal to 33.64 per cent. of carbon ; and 0.1150 of water equal to 3.31 per cent. of hydrogen ; 0.315 grm. gave 14 c.c. gas at 12° C. and 769 m.m. B. Potash column = 15.2 m.m. mercury ; equal to 13.1 normal nitrogen ; equal to 5.16 per cent. These data lead to the formula  $C_9H_7Br_2NO_2$ .

	Theory	Found
Carbon . .	33.64	33.64
Hydrogen . .	2.18	3.31
Bromine . .	49.84	48.31
Nitrogen . .	4.36	5.16
Oxygen . .	9.98	9.15
	<hr/> 100.00	<hr/> 100.00

Two further experiments for the quantation of bromine in dibromobilirubin by fusion with soda, sodic nitrate, and carbonate were made. (1) gave 46.3 per cent., (2) 47.1 per cent. Br. The flux in the last determination was washed out with warm water, and nitrate of silver added before the addition of nitric

acid. The method of fusion in an open platinum dish seems to produce a loss of bromine.

*Another preparation of Dibromobilirubin.*—This preparation gave the same relative proportion as the preceding one, and on analysis the following data; all preparations were dried at 110°.

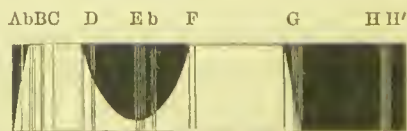
(a) 0.4502, fused with the mixture in a tube, gave argentic bromide 0.5277, equal to 49.87 per cent. of bromine. (b) 0.2314 gave 8.4 c.c. at 8° C. and 759 m.m. Bar.; potash column 13 m.m. mercury; equal to 9.0 normal nitrogen or 4.8 per cent. (c) 0.3043 grm. gave 0.3760 carbonic anhydride or 33.69 per cent. of carbonic acid; 0.0978 water, or 3.56 per cent. of hydrogen.

	Per cent	÷ Atomic weights	÷ Br. = 2
Carbon . . .	33.69	2.807	9
Hydrogen . . .	3.56	3.56	11.4
Bromine . . .	49.87	0.623	2
Nitrogen . . .	4.86	0.347	1.1
Oxygen . . .	8.02	0.501	1.6
	100.00		

Dibromobilirubin dissolves easily in absolute ether with a violet colour. The solution on spontaneous evaporation deposits violet crystals, which when thick are nearly black under the microscope. The solution must be evaporated *in vacuo* over sulphuric acid and caustic soda.



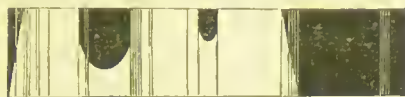
*Bromobilirubin in Alcohol and Hydrobromic Acid (colour of solution deep blue).*



*Bromobilirubin in Alcohol and Hydrobromic Acid, more dilute than the previous solution.*



AaBC D E b F G HH



*Bromobilirubin changed by Baryta Hydrate and Hydrochloric Acid (colour rose-red).*

*Action of air at 100° C. on bilirubin dibromide.*—5 grms. of bilirubin were brominated in the usual way, and it was then sought to expel the 2HBr by heating to a temperature of 100° C. in a current of dry air. This was effected in a long tube placed in a water-bath of equal length. The operation was continued for more than a fortnight, when it was found that the substance was losing its violet colour at the end of the tube in which the air entered, and becoming brown, and also that hydrobromic acid was being continuously evolved; the operation was therefore concluded. After a thorough mixing of the substance had been made the bromine was determined; 0.3238 grms. gave 0.3444 grms. argentic bromide, equal to 45.25 per cent. of bromine. Therefore dibromobilirubin at 100° C. in a current of dry air is gradually oxydised with loss of bromine as hydrobromic acid.

*Action of ether.*—From the above preparation 0.1 was taken and extracted with ether. It gave a violet-coloured solution which, when examined spectroscopically, showed an absorption stretching entirely over the yellow and nearly over the green.

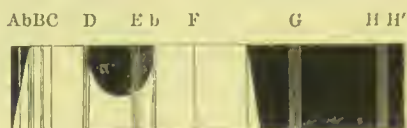
Evaporated *in vacuo* it left a non-crystalline apparently unchanged violet deposit, still soluble in ether.

*Action of sulphuric acid.*—A portion was dissolved in concentrated sulphuric acid, forming a purple-coloured solution. This was precipitated by water, and this precipitate extracted with ether; the latter took up a portion which was violet, leaving a green portion which was soluble in alcohol, first cold and then warm, giving a green solution.

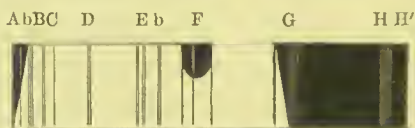
The ether solution was allowed to stand, and when shaken up with water, gave a colourless filtrate which contained free hydrobromic acid.

The alcohol solution examined spectroscopically obscured all the red, and when diluted there was a band over yellow and orange, the green being much shaded. Still further diluted

the band in red only remained, and all yellow and orange were red and a little blue appeared.



*Sulphate of Bromobilirubin in Alcohol (colour violet-blue).*



*Sulphate of Bromobilirubin in Alcohol, changed by Hyposulphite and Hydrochloric Acid.*

*Action of iodine on bilirubin.*—About 10 c.c. of a chloroform solution of bilirubin was treated with an excess of iodine in chloroform. On first mixing a darkening in colour occurred, and the iodine was added until the solution had a deep violet colour. This solution was evaporated *in vacuo* over sulphuric acid; no crystals were obtained, but only a coloured deposit in two rings, the first of which had a lighter colour than the second. When the liquid had completely evaporated, the dish was heated in a water-bath to drive off any free iodine. The residue was insoluble in ether and in water; boiling alcohol extracted a little green matter and left a residue, a little of which was dissolved in chloroform with the colour of bilirubin; the remainder of the residue, from which again alcohol extracted only a trace of green matter was left insoluble in alcohol, ether, chloroform, and benzole.

The chloroform extract left a deposit on spontaneous evaporation not strictly crystalline under the microscope.

With nitric acid it gave first a blue and then a red colour. The residue from the alcoholic extract gave a red colour only.

*Bilirubin and nitrous acid. Experiment A.*—For this preparation 0.5 grm. of bilirubin were placed in a Liebig's drying apparatus, and nitrous acid (prepared by heating plumbic nitrate in a combustion tube) allowed to pass over it; the Liebig's drying apparatus being further connected with a Gay-Lussac tube containing baryta water, in order to ascertain if any

carbonic acid resulted from the action of nitrous acid in the cold.

As soon as the nitrous vapours came into contact with the bilirubin a blackening of the powder ensued, and the reaction increased in violence every moment, much water was formed, and the whole at last took fire in the tube. There is no doubt that a total decomposition of the greater part of the bilirubin took place, carbon being deposited in the tube. Crystals (probably ammonia nitrate) were deposited on the upper surface of the drying apparatus and carbonic acid passed away.

*Experiment B.*—About 0.3 to 0.4 grm. of bilirubin was placed in a U tube with absolute alcohol and nitrous acid, prepared as before; the plumbic nitrate was previously fused to insure absence of carbonic acid. The nitrous acid was allowed to bubble through the liquid. No red fumes passed through the alcohol, which gradually became warmer and warmer until it boiled.<sup>1</sup> The bilirubin product eventually formed a deposit. Carbonic acid, aldehyd and nitrous ether were formed during the reaction, besides the bilirubin product.

*Experiment C.*—This was carried on in the same manner as the previous one; the plumbic nitrate was tested for carbonic acid and found to be free from it.

*Analyses of the resin obtained by action of nitrous acid upon bilirubin in absolute alcohol.*—The product weighed 0.4136 grm.; on combustion *in vacuo* 0.0484 gave 54 c.c. total gas at 12° C. and 763 m.m.; after potash, 5.2 c.c. at 13° C. and 754.4 B.; potash column = 3.6 m.m. mercury, mercury column = 77 m.m.; total gas normal = 49.49 c.c.

Total nitrogen = 4.83 c.c.

Total carbonic acid = 44.66

50.02 per cent. of carbon.

11.19 per cent. of nitrogen.

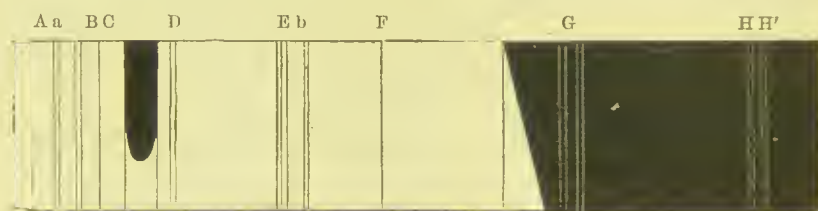
Therefore C : N = 5.2 to 1.

<sup>1</sup> *Experiment.*—*Nitrous Acid and Alcohol.*—This experiment was made with a view of ascertaining how far the reactions observed in B and C were due to the action of nitrous acid on alcohol.

The nitrous acid was passed through alcohol in a U tube; no red fumes passed, and the alcohol became warmer and warmer until it boiled. Aldehyd, carbonic acid, and nitrous ether were among the products of the reaction.

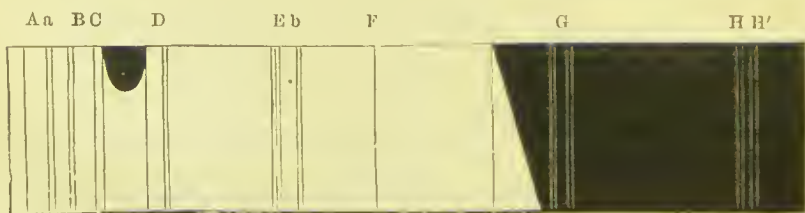
The product has consequently lost much carbon relatively to the nitrogen, or has gained nitrogen relatively to the carbon, most probably by the entrance into its constitution of a molecule of nitroxy; perhaps loss of some carbon, and entrance of nitroxy have taken place at the same time, and the product is mainly a nitrited biliverdin. Such a body would contain 48·7 per cent. of carbon and 14· per cent of nitrogen. Thus it is shown that the problem is yet far from its solution.

*Bilirubin and nitric acid. Cholocyanin.*—An ammoniacal solution of cholophæin was treated with concentrated nitric acid until a blue precipitate was formed. This was quickly isolated by filtration, and after washing with water dissolved in alcohol. It showed an absorption band in yellow. The red was shortened, the green was somewhat obscured; the first half of the blue was clear, but the second half and the rest of the spectrum entirely obscured.



*Diagram of Cholocyanin Spectrum.*

A sulphate of cholocyanin could also be obtained by the action of fuming sulphuric acid and subsequently water upon cholophæin together with various other green products insoluble in water. The very dilute watery solution showed spectrum with a feeble band on the side towards red of D, yellow obscured, red dimmed, green lively, blue only one half its ordinary length, rest obscured.

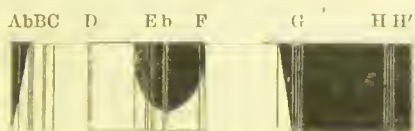


*Diagram of Spectrum of Sulphate of Cholocyanin dissolved in water.*

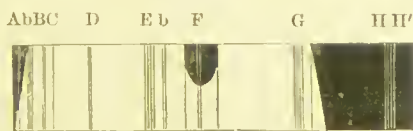


This spectrum of sulphate of cholocyanin by sulphuric acid differs from the one by nitric acid only in this, that the terminal of the band on the red side is more advanced towards red than that of cholocyanin by nitric acid, while the line nearest to yellow remains the same in both preparations.

*Influence of Sodium-amalgam upon Bilirubin and Biliverdin.*—Hydrogenised bilirubin was made by suspending pure bilirubin in water, and treating it with sodium-amalgam in excess. It was found that the action was completed in a few minutes, and did not require from two to four days as some authors state. When I continued the action of the amalgam for some days, no other products resulted than those obtained after the first half-hour. The alkaline solution was red, and on addition of hydrochloric acid deposited a rust-coloured precipitate; it dissolved almost completely in absolute alcohol, leaving a mere trace of what seemed to be unchanged bilirubin on the filter. The solution was red. It cut off the whole of the spectrum from b to the violet end. On dilution a band became detached, which had more than double the breadth of the band of hydrogenised biliverdin. A blue and violet space of the width of the



*Bilirubin transformed by Sodium-amalgam, dissolved in Alcohol (colour of solution red).*



*Biliverdin changed by Sodium-amalgam, dissolved in Alcohol (colour reddish brown).*

band also appeared. When the solution was further diluted, a narrow band remained, and the blue and violet part of the spectrum was as wide as the red, yellow and green part.

Even without the aid afforded by the great differences in bearing before the spectroscope, it is clear from the above that hydrogenised bilirubin and urochrome cannot be identical,

because the former is insoluble in water, and may be washed with large quantities of it on the filter, while urochrome is easily soluble in water, and is not precipitated from its alkaline solutions by hydrochloric acid.

A second experiment was now made with a larger quantity of material, and succeeded like the first. The new body was precipitated by acid, and after washing with water, dissolved in alcohol. A reddish-brown solution was formed, which on dilution with alcohol, became red, and ultimately light amber-coloured; it was, if at all, doubtfully and faintly fluorescent. Its spectrum showed a decided band, even when the solution was much diluted with alcohol, at the junction of green and blue, sharply defined and of deepest intensity at the green edge, intensity gradually decreasing towards blue. The amount of blue which remained by the side of the band was only about one-third of the width of the band. The band contracted on dilution exactly as the band in the first experiment.

The main quantity of the precipitated hydrogenised bilirubin was now dissolved in and boiled with hydrochloric acid. No change was observed; the solution retained its properties and remained clear. In particular none of the decomposition-products of urochrome were formed, which, had any urochrome been present, must have been formed immediately. On standing exposed to air, the solution assumed a rose-red colour.

Hydrogenised bilirubin is stated to have the formula,  $C_{32}H_{40}N_4O_7$ , and declared to be a tribasic acid. This construction is supported by only one silver-compound, which yielded 35.75 per cent. of Ag, while other preparations gave 37.1 per cent. Ag; and by one zinc-compound, which gave 14.2 per cent. Zn, while other preparations gave up to 37 per cent. of Zn. These data therefore do not afford the means for determining either the atomic weight or the basicity of the product, but seem to show that the sodium-reaction produces a variety of new products, which remain partly mixed in the precipitate, partly in the mother liquor from which it falls. For this liquid remains red, and retains a considerable quantity of a by-product.

Similar results concerning hydrogenised bilirubin were lately obtained by L. Disqué. In the reduction of bilirubin with sodium-amalgam he employed heat (which had not been employed in the former experiments), and obtained further reduction

of the hydrogenised bilirubin to a colourless matter. This shows no absorption spectrum, but when treated in chloroform, with air, it is again oxydised and transformed back into hydrogenised bilirubin. These experiments of Disqué were made upon small quantities in test tubes only, and did not lead to the isolation of definite compounds.

*List and formulæ of definite biliary colouring matters, their derivates and compounds isolated in these researches.*—The biliary colouring matters and their compounds, as evolved by my researches, are now the following:—

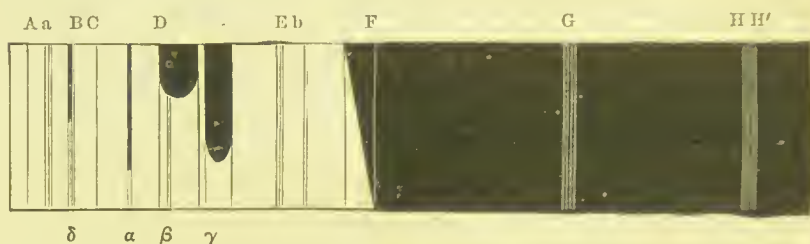
Crystallised bilirubin .	$C_9H_9NO_2$
Neutral silver salt .	$C_9H_8AgNO_2 + H_2O$
Basic silver salt .	$C_9H_7Ag_2NO_2$
Neutral baryum salt .	$C_{18}H_{16}BaN_2O_4 + 2H_2O$
Half acid „	$C_{18}H_{16}BaN_2O_4 + C_9H_9NO_2 + 2H_2O$
Neutral calcium salt .	$C_{18}H_{16}CaN_2O_4 + 2H_2O$
Half acid „	$C_{18}H_{16}CaN_2O_4 + C_9H_9NO_2 + 2H_2O$
Half acid zinc salt .	$C_{18}H_{16}ZnN_2O_4 + C_9H_9NO_2 + 2H_2O$
Basic lead salt .	$C_9H_7PbNO_2$
Dibromo-bilirubin .	$C_9H_7Br_2NO_2$
Hydrobromo-bilirubide	$C_9H_8BrNO$
Hydrobromo-bilirubide bilirubin .	$C_9H_8BrNO + C_9H_9NO_2$
Cholothallin .	$C_9H_{11}NO_3$
Bilifuscin .	$C_9H_{11}NO_3$
Biliverdin .	$C_8H_9NO_2$
Bromobiliverdin .	$C_8H_8BrNO_2$

*Notes on abnormal biliary pigments and their spectra.*—The following substances are natural products of diseased processes.

*Cholonematin.*—The residue from the alcoholic extract of colouring matter from human gallstones was dissolved in ether. The solution appeared green in reflected light, brown in transparent dilute solution. The latter showed a remarkable spectrum of four bands. Of these two were thread-like (hence the name), thin, like sun-lines; two broader ones were in green and blue.

Green shaded. The fine lines are clearly visible with the sulphide of carbon prism only, but are fused together when seen

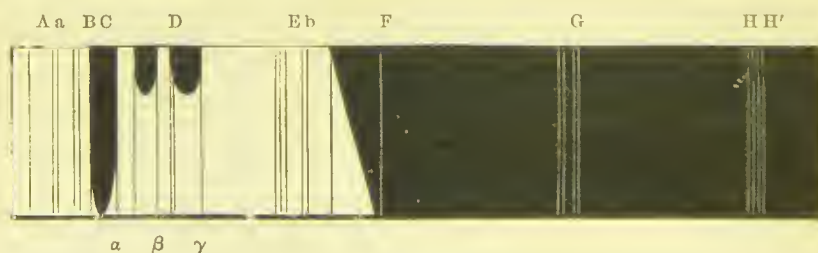
in the magnifying spectroscope with flint-glass prisms. In the latter red extends apparently uniformly to  $\alpha$ , and is there suddenly cut off. Blue is cut off entirely, even in very dilute solution, the four bands remain to the end,  $\delta$  becoming feeblest.



*Diagram of Spectrum of Cholonematin.*

Addition of sulphuric acid makes  $\delta$  and  $\alpha$  very feeble. The spectrum has some similarity to that of xanthophyll (from chlorophyll of grass). The alcoholic solution of ether extract of bilifuscin from human gallstones before precipitation with ether, on comparison showed no bands. Most similar to cholonematin is the spectrum of fluopittin, described in my researches on the decomposition products of the albuminous substances. But in this latter the wider of the two thread-like lines is situated towards the red end, and the thinnest one inside of it, the reverse being the case in cholonematin.

*Boviprasin*, a green colouring matter from gallstones of *ox.*—The alcoholic extraction from the gallstones left on



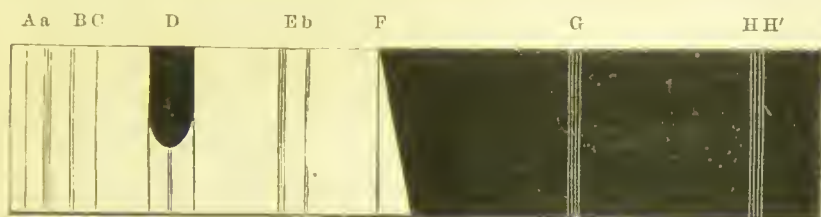
*Diagram of Spectrum of Boviprasin.*

evaporation a resinous residue which dissolved in ether was submitted to spectral analysis. It showed three bands.

Seen in the non-magnifying spectroscope,  $\beta$  is divided, a phenomenon not caused by the interference of the sodium line, as this latter is on the band  $\gamma$ .

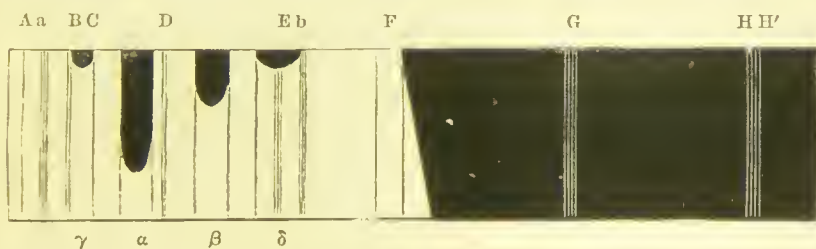


*Bovifuscopittin*.—This resin was extracted from ox gallstones by alcohol. It was treated with caustic potash, precipitated by hydrochloric acid, and dissolved in ether. It did not crystallise. Fatty acids were then removed by precipitation with lime, and the resin was dissolved in alcohol. The solution was brownish yellow to red, without any green tinge whatsoever. Its spectrum presented a single band overlying the D line.



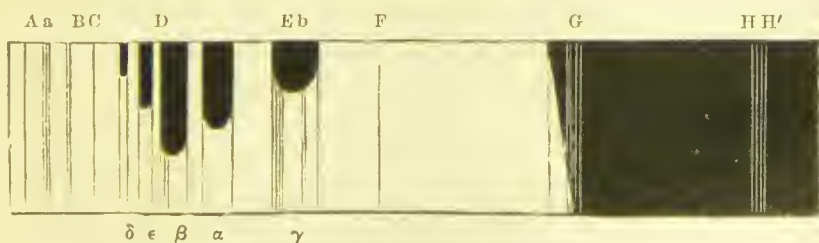
*Diagram of Spectrum of Bovifuscopittin.*

*Muscoprasin*.—This resin is extracted from ox gallstones by means of alcohol. It has a green colour in the solid state, and retains it on solution in alcohol. In the spectrum it shows bands of great distinctness, one in red being very black. The resin has a particular musk flavour.



*Diagram of Spectrum of Muscoprasin.*

*Ethochlorin*.—The first ether extraction of ox gallstones yielded a green-coloured substance, which in its second ethereal solution showed the following spectrum :—



*Diagram of Spectrum of Ethochlorin.*

In the magnifying spectroscope with flint-glass prism the band  $\delta$  is hardly seen. Red is suddenly cut off, and is then cut off a second time. The margin of the band  $\delta$  towards yellow, and that of the band  $\varepsilon$  towards the red end, are apparently washed away. This is the most complicated spectrum met with amongst biliary matters, and should be compared to that of cruentin.

## XXI.

*SPERMATIN, A NEW ORGANIC BASE IN ANIMAL ORGANISMS. (Summary.)*

THIS substance was discovered by Ph. Schreiner (*Liebig's Ann.* 194, 1878, 68), but its phosphate had been known under the shape of microscopic crystals of peculiar shape occurring in animal tissues and liquids for twenty-five years. The discovery thus illustrates the great use of microscopic observation as a pioneering power; peculiar forms are seen perhaps under anomalous conditions, and noted; they are then again noticed on a field of wider observation, and ultimately searched for. The search then leads to the discovery of materials from which the microscopic objects can be isolated in sufficient quantity for being subjected to chemical inquiry.

Charcot and Robin (*Compt. rend. de la Soc. de Biologie*, 1853, 49) were the first to observe the crystals in the spleen of a leukæmic subject. Charcot in 1856 found them in the sputa from a case of emphysema with catarrh. From this the crystals have frequently been termed 'Charcot's crystals.' Förster (*Atl. d. microscop. Anat.* 1859, Tab. 33, fig. 4, p. 67 of text) saw them in the bronchial discharge of a man suffering from temporary bronchitis, in a so-called mucinous tissue tumour of the optic nerve, and in the inspissated mucus of an enlarged biliary duct, found them insoluble in ether, and surmised that they consisted of a substance related to mucus. Harting (*Das Microscop.* 1859, 458; fig. 182, C.) saw the crystals in the sputa in chronic bronchitis, observed that they were insoluble in water, alcohol, and ether, but soluble in acetic, hydrochloric, and nitric acid, and interpreted them as calcic phosphate. The crystals were again observed by Charcot and Vulpian (*Gaz. hebdom.* 7 (1860) 47) in the blood from different parts of the

body of a female dead from leukæmia. In this case it was observed that the crystals only began to make their appearance a day after the post-mortem examination, and that their number kept on increasing during the following days. These authors found the crystals brittle, insoluble in cold water, alcohol, ether, chloroform, glycerin, watery and alcoholic solution of iodine, but soluble in warm water, acetic, tartaric, lactic, sulphuric, hydrochloric acid, and in solutions of potash, soda, and ammonia, and supposed that they consisted of an organic body. The crystals were studied by White (*Boston Med. and Surg. Jour.* Nov. 28, 1861) and termed by him 'leucosin;' he found them in every case of leukæmia. In 1862 they were noticed by Wagner (*Arch. d. Heilk.* 3, 379) in the blood of the portal vein of an anæmic woman who had died shortly after her delivery of a child, suddenly and without any previous illness. In 1864 the same crystals were described and figured by Friedreich (*Arch. f. path. Anat.* 30, 382) recognised as identical with those described by Förster, and declared to consist of tyrosin. They were found in fibrinous bronchial sputa of a woman æt. 42. In the same year Huppert (*Schmidt's Jahrb.* 124, 147) observed that the crystals do not rarely occur in the blood of leukæmic patients.

In 1865 Böttcher (*Arch. f. path. Anat.* 32, 525) published a paper 'on colourless crystals of an albuminous body, obtained from human sperma.' He found them forming in the plasma of the sperma when it was allowed to dry spontaneously. Subsequently he observed them on the surface of old pathological-anatomical preparations, and was able to obtain them from egg-albumin, and believed that he had confirmed their albuminous nature. In 1866 Neumann (*Arch. f. microscop. Anat.* 2, 507) found the crystals in the blood taken from the body of a leukæmic subject; the formation of the crystals began a few hours after the post-mortem examination, and increased during the following days to such an extent, that every drop of blood contained considerable numbers of them. In 1868 Eberth (*Arch. f. pathol. Anat.* 43, 8) noticed the crystals in connection with leukæmia, and in 1869 Neumann (*Archiv. d. Heilk.* 10, 220) found them in nearly all dead human bodies which he examined, in the normal marrow of the bones. In a great



number of cases of bronchial asthma the crystals were found by Leyden (*Arch. f. pathol. Anat.* 54, 324 and 346) and declared to be specific products of that disorder. In 1875 crystals were found by Zahn (*Arch. f. pathol. Anat.* 62, 107) in the curdled blood of a frog the subject of experiments upon thrombosis, and found to be identical with those observed by Brondgeest (*Nederl. Arch. voor Genees. en Naturk.* 5, 1870, 378) in the blood of frogs which had died from the effects of frost. In 1876 a case of leukæmia gave the opportunity for the observation of these crystals in the bone-marrow and in the mesenteric glands to Lauenstein (*Deutsch. Archiv. f. klin. Med.* 18, 1876, 122). In the same place Zenker gave a summary of the observations concerning these formations, which had frequently come under his eyes in cases of splenic leukæmia and in the sputa of bronchial asthma.

Instructive are the various surmises which the observers of these crystals, or their commentators, and particularly the writers of systematic treatises, made concerning their nature. Some declared them to be tyrosin; one author believed this to be an indubitable certainty (Huber, *Arch. d. Heilk.* 18, 1877, 485); a third declared it to be a substance related to mucus, a fourth to mucin; a fifth again believed in their albuminous nature; a sixth held them to be related to vitellin; a seventh likened them to yolk-plates, and crystals of aleurone from plants; an eighth declared them to be magnesian phosphate; a ninth calcic phosphate. Schreiner set all these surmises at rest by showing that the crystals were the phosphate of an alkaloid hitherto unknown, and widely distributed in the animal economy.

*Mode of obtaining the phosphate of spermatin, (a) from sperma.*—Fresh sperma, washed out of linen by warm water, is evaporated to dryness, boiled with alcohol, and allowed to cool and stand for several hours. The mixture is now placed on a filter, and the residue on this is washed and dried. This residue containing the spermatin phosphate is now triturated and extracted with warm water to which a few drops of ammonia have been added; only traces of the albuminous substances pass into solution and remain so, while the new salt on slow evaporation of the solution crystallises again in its peculiar forms. In a

quantation the dry residue of sperma was found to yield 5.237 per cent. of these crystals.

(b) *from organs of animals*, e.g. calf's liver, calf's heart, bull's testicles, the crystals are obtained by letting the organs stand immersed in alcohol in well-closed vessels. After several months of standing, slender double pyramids, sometimes five

FIG. 1.



FIG. 2.



millimetres in length, are deposited on the surface of the immersed organs. The crystals are scraped off, the matter thus isolated is extracted with warm water mixed with a few drops of ammonia, and the filtrate on evaporation furnishes the former crystals but of smaller size.

*Forms of the crystals.*—Haushofer described them as combinations of prismatic with pyramidal forms which mix with each other so as to present spindle-shaped bodies with convex surfaces. The prism fig. 1 is so to say wiped out as regards edges which are diagonal to each other, as is shown in fig. 2, where the lower edges of the anterior contiguous narrow and broad perpendicular planes of the prism  $\infty$  P have been smoothed off, as have the diagonal upper edges on the hindmost side of the crystals. The crystals combine so as to form crosses and rosettes.

*Properties of the crystals.*—After repeated crystallisation from hot water to which a few drops of ammonia have been added, the crystals, from whatever source, exhibit the following properties: they are brittle, transparent, colourless, insoluble in alcohol, ether, chloroform, salt-water, watery and alcoholic solution of iodine, almost insoluble in cold, little soluble in hot water, easily soluble in dilute acids, including chromic and nitric, and in caustic and carbonated alkalies, including ammonia. The solution of the crystals in water acts on sensitive red litmus paper just like common sodic phosphate; the paper becomes blue, but the blue colour disappears when the paper dries in air. When heated on platinum the crystals at a temperature little above  $100^{\circ}$  become cohesive and assume a yellow colour; at  $170^{\circ}$  they

fuse, and the fused mass sets again on cooling; on the application of higher degrees of heat, ammoniacal vapours are emitted, and a black, shining, almost incombustible residue of charcoal is left, which has an intensely acid reaction. On raising the heat the charcoal disappears and a glassy shining spot of phosphorus-platinum is left on the platinum employed. The crystals when burned on a platinum-wire colour the extreme point of the blowpipe flame green. Mixed with the magnesia test for phosphoric acid, a precipitate of the double salt is immediately obtained.

*Water of crystallisation.*—The crystals lost in a partial vacuum over sulphuric acid 16·58 per cent. and on subsequent heating to 100° in an air bath they lost again 4·57 per cent., together 21·15 per cent. of water of hydration and crystallisation.

The same crystals recrystallised from hot water lost again 16·667 per cent. *in vacuo*, and 4·545 per cent. at 100°, together 21·212 per cent. of water.

*Phosphoric acid.*—A portion of the crystals dried at 100°, dissolved in water with a drop of ammonia, and treated with magnesia mixture, gave pyrophosphate of magnesia equal to 35·399 per cent. of phosphoric acid in the crystals. A second quantation gave 35·126 per cent.  $P_2O_5$ .

*Nitrogen.*—Ignited with soda lime the crystals gave ammonia corresponding to 14·03 per cent. of nitrogen in the salt.

From these data it follows that the salt at 100° loses three molecules of water of crystallisation, and that it contains two atoms of nitrogen upon one of phosphorus.

*Isolation and properties of spermatin.*—The phosphate is decomposed accurately with baryta water, and the filtrate is evaporated in thin layers on the water-bath. The base then remains as a colourless viscous varnish-like mass, which on cooling crystallises. If the solution is evaporated in larger quantities and in thicker layers, a thick syrup is obtained which crystallises only partially at the margins, even on standing for several days over sulphuric acid. The crystals disappear quickly on exposure to the air, and the viscous syrup becomes more fluid, absorbing both water and carbonic acid. The base is soluble in absolute alcohol, and crystallises from this solvent easily on

evaporation. It is almost insoluble in ether, and seems to be changed by this solvent, being transformed into a brown mass, which after removal of the ether becomes only gradually soluble in water.

On adding to the base in the syrupy or carbonated state some phosphoric acid, or ammoniac phosphate, the crystals of the phosphate above described and figured are at once obtained. Spermatin therefore expels ammonia from its compounds, and its phosphate is distinguished by a great tendency to crystallisation. It is not changeable when exposed to the air. Heated on platinum it evolves thick white fumes, which have only a weak ammoniacal odour.

The watery solution of spermatin, which has a strongly alkaline reaction, shows also the following bearing with reagents: (1) On being warmed with caustic potash or soda it evolves ammonia; (2) with zinc chloride it gives a white flaky precipitate which is soluble in hydrochloric acid; (3) with watery solution of tannin it gives a white flaky precipitate; (4) with argentic nitrate it yields a white precipitate soluble in nitric, sulphuric and acetic acid; (5) corrosive sublimate produces only a turbidity in its solution; (6) with auric trichloride the base yields at once a golden yellow precipitate, which becomes gradually crystalline; (7) with platinic tetrachloride the base yields no immediate compound, but after some time delicate plates are obtained; (8) phosphomolybdic acid yields a yellowish precipitate with spermatin; (9) phosphotungstic acid gives a white flaky precipitate, which is soluble in ammonia, potash, and soda, but insoluble in hydrochloric, nitric, sulphuric, phosphoric and acetic acid. With iodide of potassium and with neutral and basic lead acetate the base gives no reaction.

*Mode of extracting the base from tissues and organs.*—Phosphotungstic acid may be employed for isolating the base from the parts of healthy animals, such as liver, spleen, lungs and blood of cattle, and from the parts of bodies which have died from disease. The parts are minced, boiled with water and a little acetic acid, and the filtered liquid is treated with basic plumbic acetate, an excess of this reagent being avoided. The filtrate from this lead precipitate is freed from lead by



hydrothion, boiled, and after suitable concentration treated with phosphotungstic acid. The precipitate is washed with water to which sulphuric acid has been added, and then decomposed by baryta water in the hot state. Any excess of baryta is removed by carbonic acid. The concentrated liquid may contain other alkaloids besides spermatin; from these the latter is best separated by the addition of phosphoric acid, care being taken to keep the mixture as neutral as possible on account of the easy solubility of the phosphate in acids and alkalies. When bases are present which retain baryta in combination, the isolation of the phosphate of spermatin from the barytic phosphate has to be effected by recrystallisation from hot water.

*Hydrochlorate of spermatin.*—The watery solution of free spermatin neutralised with hydrochloric acid gives on slow evaporation on the water-bath the hydrochlorate as six-sided prisms united in tufts; the salt is not changed by air, insoluble in ether, almost insoluble in alcohol, soluble in spirit, and very easily soluble in water. Addition of absolute alcohol to the watery solution causes the salt to crystallise in anhydrous small crystals, which on analysis yield numbers leading to the formula  $C_2H_5N, HCl$ . This requires in 100 parts

C	.	.	30.204
H	.	.	7.551
N	.	.	17.618
Cl	.	.	44.626

These data show that the phosphate of spermatin dried at  $100^\circ$  does yet contain a molecule of water of crystallisation, which cannot be expelled without decomposing the salt.

*Platinic double salt.*—After addition of platinic tetrachloride to spermatin hydrochlorate a double salt is gradually produced, which crystallises in prisms.

*Auric double salt,*  $C_2H_5N, HCl, AuCl_3$  (Au=51.33 per cent., Cl=37.146 per cent.) The hydrochlorate gives with auric terchloride immediately a voluminous precipitate, consisting of shining, golden yellow irregular plates. When freshly precipitated this salt is easily soluble in ether, alcohol, and water; but when dried and exposed to air it loses its solubility in

water in part, and when the redissolved part is allowed to stand, a further precipitate of insoluble matter takes place.

When the gold salt dissolved in water is treated with magnesium, the odour of fresh human sperma is at once produced. From this it is probable that the odour of human sperma is due to the presence of a derivate of spermatin. The odour of sputa, which not rarely resembles that of sperma, is probably also due to the presence of this derivate.

When gold and chlorine are removed from the salt by Scheibler's process (*Ber. Deut. Chem. G.* 2, 297), and the filtrate from the silver chloride is freed from silver by hydrothion, the filtrate from the silver sulphide gives again a precipitate with phosphotungstic acid, which is not identical with the precipitate given by this reagent with the original base; the body isolated by the baryta process is a colourless alkaline, inodorous syrup, but cannot be brought to crystallise with either phosphoric or hydrochloric acid.

When gold and chlorine are removed from the gold salt by hydrothion and silver oxyde, the alkaline solution retains silver, and on evaporation on the water-bath forms a silver mirror on the walls of the dish; on further evaporation in a new dish a brown syrup, free from silver, is obtained, which does not crystallise with either hydrochloric or phosphoric acid.

## XXII.

*CHEMICAL SURGERY, WITH SPECIAL REFERENCE TO  
THE ANTISEPTIC PHARMACOPŒIA.<sup>1</sup> (Review.)*

IN the first part of this 'Guide' the author gives a description of the condition of affairs in the surgical department of the hospital at Munich before the introduction of the antiseptic treatment of wounds. Pyæmia was permanent, and nearly all cases in which amputation had been performed perished by it. In 1872 hospital gangrene appeared, and became both severe and frequent, so that in 1874 80 per cent. of all wounds and ulcers were affected by it; corroded arteries and mortified bones led to mutilations, and frequently to death. Erysipelas appeared upon almost every patient; every patient became at least once, frequently twice, the victim of hospital gastricism; a first reunion, or union of wound by prime intention was never observed before 1875.

All these evils are now removed by the introduction of Lister's antiseptic treatment of wounds. Erysipelas, phlebitis, hospital gangrene, and pyæmia have disappeared; the wounds resulting from amputations and plastic operations heal by first reunion; the mortality in the clinic has been diminished to one-half its former proportions. This very striking success causes the author to become a strong advocate of the antiseptic method, and he fills the first part of his 'Guide' with arguments to the effect, that every surgeon is in duty bound to know and to practise the antiseptic method.

In the second part of this 'Guide' Nussbaum gives a concise description of the apparatus and chemical preparations required

<sup>1</sup> Prof. Dr. J. N. Ritter von Nussbaum, *Guide to the Antiseptic Treatment of Wounds, particularly to the Method of Lister*. Third edition. Stuttgart, 1879.

for the successful application of the method, from which we quote the following practical details.

1. *Carbolic acid solution of 5 per cent. strength.*

℞ Acid. carbolic. crystallisat. puriss. 50·0.  
Aq. destillat. 950·0 (grammes).

This solution is used for the ablution of the hands of the operator and of the assistants before every operation and before every dressing. The surfaces upon which an operation has to be performed are also washed with it, and the area surrounding every ulceration. The surgical instruments are placed in this solution before and during the operation, canulas and catheters are rinsed with it both in and outside.

The apparatus for producing the steam spray is filled with it. The steam in dispersing the solution dilutes it to the extent that the condensed spray represents a solution of carbolic acid in water of about  $2\frac{1}{2}$  per cent. strength.

This watery solution of carbolic acid has the disadvantage that it causes roughness and even painful excoriation of the skin to some who have to operate much and dress frequently. This drawback is avoided by the use of carbolised vaselin, an ointment consisting of 90 grms. of vaselin and 10 grms. of carbolic acid; it can be distributed over the whole of the hands by friction, disinfects perfectly, and does not produce any roughness of the skin of the hands. This roughness is always attended by a certain loss of sensibility in the fingers, and is therefore to be dreaded by the surgeon, who requires the utmost sensibility in his finger-tips in daily practice.

Vaselin is a paraffin obtained from petroleum, semi-solid at the ordinary temperature, fusing at about  $35^{\circ}$ , and boiling at about  $200^{\circ}$ . It has been used since 1875, for the compounding of ointments, instead of fat.

2. *Carbolised water, or watery solution of carbolic acid of  $2\frac{1}{2}$  per cent. strength.*

℞ Acid. carbolic. crystallisat. puriss. 25·0.  
Aq. destillat. 975·0.

This water is used to moisten dressings, wash wounds; it is also used in the spray-producer.



3. *Carbolised oil of 5 per cent. strength.*

℞ Acid. carbolic. crystallisat. puriss. 5·0.  
 Olei olivar. pur. 95·0.

With this oil catheters, specula, fingers, and hands are anointed before use.

4. *Carbolised oil of 10 per cent. strength.*

℞ Acid. carbolic. crystallisat. puriss. 10·0.  
 Olei olivar. pur. 90·0.

This mixture is used to drench lint, which it is intended to place into deep wounds, such as arise after carious bones have been scraped or cancerous tumours have been scooped out.

5. *Salicylic emulsion.*

℞ Acid. salicyl. crystallisat. 5·0.  
 Aq. destillat. 95.

This preparation is actually salicylic acid suspended in a saturated solution of the acid (5 grms. of the acid require 1500 grms. of water for solution). It is used for moistening dressings, when it is intended to leave them undisturbed for a longer time, and is in such cases preferred to carbolised water. It must be shaken up before use.

6. *Solution of zinc-chloride of 8 per cent. strength.*

℞ Zinci chlorid. 8·0.  
 Aq. destillat. 92·0.

This solution is used to make wounds and ulcers into which septic ferments have penetrated, again aseptic.

7. *A steam spray-producer* with spirit or gas lamp.

8. *Silk or protective*, a thin green oil-silk, which is covered with a mixture of 1 part dextrin, 2 parts amylon, and 16 parts of a watery solution of carbolic acid of 5 per cent. strength.

This protective is used to cover up wounds and ulcers, to which it adapts itself easily, owing to its pliability. The covering of carbolised paste is necessary to insure the destruction of all ferments which may possibly adhere to the oil-silk.

9. *Antiseptic gauze*.—Bleached or unbleached cotton gauze is cut into pieces 6 metres long and 1 metre broad. They are

heated in a tin case, surrounded by boiling water, during from 2 to 3 hours. The tissue is now spread out and impregnated with a hot mixture consisting of 1 part crystallised carbolic acid, 5 parts rosin, 7 parts solid paraffin. The rosin is intended to fix the carbolic acid; the paraffin is added to remove the adhesiveness of the carbolised rosin. The gauze thus impregnated is again placed into a tin box and pressed for an hour or two, to ensure that the mixture may penetrate all the fibres.

Of this gauze 6 or 8 layers of suitable size are dipped into carbolised water, wrung and laid directly upon the silk covering the wound. This piece of gauze has been termed the lost gauze. Over this are laid 8 layers of dry gauze, which must overlap the wound in all directions. Over this is laid a piece of impermeable caoutchouc tissue or gutta-percha paper. The entire dressing is fastened with rollers of the prepared gauze.

10. *Caoutchouc tissue*, or mackintosh, made of cotton stuff and caoutchouc, or gutta-percha paper, is used to cover the gauze-dressing, and prevent the secretion of the wound from coming into visible contact with the outer air; the secretion is thus compelled to soak into the gauze and remains unaffected by ferments.

11. *Catgut*.—The catgut occurring in trade is made from the small intestine of sheep. It must be prepared by submersion during two or three months in an emulsion consisting of 5 parts of olive oil and 1 part of liquid carbolic acid (the crystallised carbolic acid after addition of 10 per cent. of water becomes permanently liquid). The catgut thus prepared is kept in carbolised oil (3). It is used to tie arteries or veins; the ligatures are cut short and allowed to remain in the wound; they unite with the tissue of the scar as if they were living tissue. Catgut can also be used for sutures. Such do not require to be removed, as the portions within the tissue are absorbed, while those outside fall off. Latterly a kind of catgut prepared with chromic acid has been proposed.

12. *Salicylated cotton-wool*.—Cotton-wool is freed from fat by boiling with caustic ley, washed and dried. This is now dipped, in thin layers, into a solution, consisting of 1 kilogramme of salicylic acid, one litre of spirit of 0.083 spec. gr., and 60 litres of water of 80° C. temperature. It is allowed to remain

for some hours, taken out, allowed to drain without pressure, and then dried on boards.

13. *Salicylated jute*.— $2\frac{1}{2}$  kilogrammes of jute (or tow) are steeped in a mixture of 500 grms. glycerin and  $4\frac{1}{2}$  kilogrammes of water, containing 75 grms. of salicylic acid in solution.

These salicylated materials are used for adapting the dressings very closely to the skin surrounding the wound and dressings. Nussbaum thinks it possible that salicylic acid might destroy ferments which carbolic acid might leave untouched, and *vice versâ*, and that both disinfectants might give the best results when employed at the same time.

14. *Sponges* prepared as usual, are kept ready for use in a watery carbolic acid solution of 5 per cent. strength. They are frequently used to stanch bleeding by gentle compression. The bloody sponges are washed with soap-water two or three times, then with tepid water ten times, then dipped in dilute carbolic or hydrochloric acid, washed again in water, and preserved again in carbolic acid water.

15. *Drainage tubes* of caoutchouc, or small bundles of horse-hair are placed into the lowest angles of wounds to facilitate the discharge of the wound secretion. These articles are washed with soap-water and disinfected with solution of carbolic acid.

16. *Antiseptic silk*.—Silk thread of suitable size is steeped in a hot mixture of 1 part of carbolic acid, and 10 parts of bees'-wax. The excess of the wax is removed by drawing the threads through a cloth.

This antiseptic silk is useful for sutures, and for ligatures; it may be left in the wound, which heals over it without difficulty, although the silk, unlike the catgut, is said not to be absorbed. It rivals silver wire and carbolised horsehair for some kinds of sutures.

17. *Salicylated water*.—A solution of 3 parts of salicylic acid in 900 parts of water. This water is used to wash wounds in cases where the solution and preparations of carbolic acid produce a kind of blood-poisoning, which is indicated by indisposition of the patient and dark green colour of his urine. It is also placed in the spray-producer, to feed the spray during operations on children. For the young are easily affected by carbolic acid, and sometimes severely and dangerously so; the

carbolic preparations have therefore to be used in their case with great caution.

18. *Boracic acid water*.—35 grms. of boracic acid are dissolved in 965 grms. of water. This solution has a long-lasting antiseptic effect, and is free from the objections which arise in the case of children to carbolic acid preparations. The solution is used to moisten lint with it, and wash wounds dressed with such lint. The solution is also useful for injections in gonorrhœa, in blenorrhœa of the bladder, and for other purposes.

19. *Boracic lint*.—Lint impregnated with boracic acid is a good dressing for ulcers, scalds, and burns; it is covered over with caoutchouc tissue, or gutta-percha paper.

20. *Boracic ointment*.

℞ Acid. boric. pulverisat.  
 Ceræ albæ āā 10.  
 Olei amygdalar. dulc.  
 Paraffin āā 20.  
 Ft. unguent.

This ointment is spread on disinfected calico in thin layers, and this is placed over wounds of the skin united by sutures; it is then fixed by gauze dipped in collodium. Such a dressing is very useful in cases of operation for harelip.

In the third part of his 'Guide' Nussbaum describes in detail and exemplifies by cases the methods of applying these preparations. In the fourth part he advocates the earliest possible use of the method in wounds occurring to soldiers in the course of warfare, and recites instructive examples of its success in the hands of Russian surgeons during the Russo-Turkish war.

Although in some places the case of the antiseptic method is overstated, the examples amply justify the elated tone of the author. Some details no doubt exemplify conditions specially Bavarian, and would perhaps not be met with in any other part of Germany, the Wendish districts excepted. On the whole however the 'Guide' of Prof. von Nussbaum is a valuable, interesting, and concise exposition of the antiseptic method as applicable to most human conditions, and Professor Lister may be congratulated on having found so ardent and so able an expositor of his discoveries and views.



## XXIII.

*HISTORICAL RETROSPECT ON EARLIER, AND CRITICAL  
CONSIDERATION OF CONTEMPORANEOUS, RE-  
SEARCHES ON BILIARY PIGMENTS.*

THE colouring matter of bile has repeatedly attracted the attention of chemists and physicians. The first attempt to extract it from bile was made by Berzelius. He obtained a yellow substance which was soluble in ether, contained soda, and was named bilifulvin. He also endeavoured to extract the green colouring matter, by combining it with baryta. When the green precipitate was decomposed with hydrochloric acid, biliverdin was left. At a later period Scherer attempted (*Ann. Chem. und Pharm.* 53, 377) to isolate the yellow colouring matter of bile contained in the urine of jaundiced persons, and performed the elementary analysis of a substance obtained from such urine, as also the analysis of a colouring matter which by a different proceeding had been obtained from gallstones. The colouring matter from gallstones was also subjected to elementary analysis by Hein and Marchand (*Physiol. Chem.* 25). As the substance analysed by these chemists was yet very impure, it yielded no satisfactory results. Some addition to our knowledge regarding the nature of this substance was made by the researches of Heintz (*Poggend. Annal.* 84, 106). He purified the colouring matter from gallstones by solution in carbonate of soda, and separated by means of alcohol the brown from the green modification. He further investigated the metamorphosis of the brown into the green colouring matter under the influence of alkalis and air, and upon the basis of several elementary analyses he conceived this metamorphosis to consist in an oxydation. One equivalent of biliphæin absorbed, according to the theory of Heintz, one equivalent of oxygen, and then

split up into two equivalents of biliverdin ( $C_{32}H_{16}N_2O_8 + O = 2(C_{16}H_8NO_5)$ ). After Valentiner (*Günsburg's Zeitschrift N. F.* 1, 46, 1859) had extracted a crystallised matter from gallstones by means of chloroform, which matter he announced to be hematoidin, Städeler (Frerichs, *Klin. d. Leberkr.*) applied the same solvent upon gallstones and obtained crystallised biliphæin, to which in accordance with his analysis, he attributed the formula  $C_{18}H_9NO_4$ . At a later period, however, Städeler published (*Mittheilungen aus dem chem. Laborator. in Zürich*, 1864) a more extensive paper on the colouring matters in human gallstones, in which, based upon new elementary analysis and one atomic weight determination, he attributed the formula of  $C_{32}H_{18}N_2O_6$  to the red colouring matter of bile, and termed it bilirubin. He observed its transformation into biliverdin, as described by Heintz, and formularised the process, without having performed any additional analysis, thus, that one equivalent of bilirubin by the absorption of two equivalents of water and two equivalents of oxygen passed into biliverdin, to which latter he attributed the hypothetical formula  $C_{32}H_{20}N_2O_{10}$ . He further distinguished bilifuscin, which was obtained directly from gallstones, and not produced from bilirubin, but placed in relation to this substance. Its empirical formula was given as  $C_{32}H_{20}N_2O_8$ , thus being distinguished from bilirubin by exceeding its formula to the amount of 2 HO. But by solution in alkali it yielded no biliverdin. Städeler further distinguished biliprasin, which was also extracted ready formed, not produced artificially, and on analysis yielded figures from which it was possible to calculate the formula  $C_{32}H_{22}N_2O_{12}$ , distinguished from the hypothetical biliverdin by an excess of 2 HO, and a colour test of its spirituous solution with caustic ammonia. Biliprasin was not obtained from biliverdin; no processes were indicated by which both substances could be separated. (In the foregoing formulæ C=6, O=8.) Already, in the year 1861, I communicated to the British Association for the Advancement of Science some researches on the metamorphosis of the colouring matter of bile by oxygen and nitrous acid, which afterwards were embodied with my work on Gallstones (London, 1862). I then published a new theory of bilirubin and its salts, based upon the comparison of many

preparations, and nearly fifty elementary analyses of these preparations, and of eight different combinations with metals, in the *Tenth Report of the Medical Officer of the Privy Council*, 1867, pp. 240 to 251 and *Journ. f. pract. Chemie*, 104 (1868), 4. This was made the subject of some hypothetical considerations by Städeler. They are to be found in the 18th volume of *Gmelin's Handbook* by Kraut, English edition, pp. 75 and 76, and are stated by the editor to be taken from an epistolary communication to him by Städeler.

It is first to be remarked that the analyses of crystallised bilirubin formerly given by Städeler, and published in Frerichs' *Handbuch der Leberkrankheiten*, are not given in Kraut's summary, nor is there any reason given either by Städeler or Kraut for their rejection or omission. They fully coincide with my own analyses and theory, and I, therefore, claim them in my support, and against Städeler's theory.

In his epistolary communication Städeler does not adduce a single new fact or analysis, nor does he controvert any of my facts and analyses, but he puts upon the whole of them a new construction, by means of which he endeavours to bring them into some kind of harmony with his theory of bilirubin. He doubles his formula of bilirubin, assuming it to be  $C_{32}H_{30}N_4O_6$  (here, and in the rest of the formulæ of this article  $C=12$ ,  $O=16$ ), and to contain six atoms of hydrogen replaceable by metals. He gives formulæ for all my salts according to that hypothesis; I quote, as examples, the basic silver salt,  $C_9H_7Ag_2NO_2$ , which in its new dress appears as  $C_{32}H_{30}Ag_6N_4O_6$ , and the neutral silver salt  $C_9H_8AgNO_2 + H_2O$ , which in its new attire becomes  $C_{32}H_{33}Ag_3N_4O_6$ . In the half-acid baryum, calcium, and zinc salts two atoms of hydrogen are supposed to be replaced by one atom of metal, thus:  $C_{32}H_{34}BaN_4O_6$ . This hypothesis has no foundation in fact. Not a single formula of Städeler's, and not a single element of any formula, can be derived from my analyses. The quantities of metals, as calculated by Städeler for his formulæ, are all from one to six per cent. below the quantities found by me. To this I must add that the facts regarding Städeler's lime-salt (the only salt he ever investigated, and that only by one calcium estimation) are misstated in the translation. Städeler found in his lime-salt 9.10 per cent. of

lime, i.e. calcium oxyde, and not as there stated 9.10 per cent. of Ca, i.e. calcium metal. It was this only lime determination which led Städeler to his second theory of bilirubin, and as he did not know of two classes of salts, which were first discovered by me, this atomic weight determination led him, according to my explanation of it, into error. It must be pointed out that all the figures given on p. 76 of the translation marked 'according to Städeler' are not results of analyses, but merely of calculations on paper by the light of the hexabasic hypothesis. I hold the hypothesis of Städeler to be not merely not proved by facts, but to be directly disproved by all my analyses, without exception, and more particularly by the brominated compound of bilirubin. A hexabrominated bilirubin, according to Städeler's hypothesis, would require 45.8 per cent. Br, whereas the dibrominated bilirubin of my formula requires and actually contains 49.8 per cent. Br.

A number of chemists have operated upon bilirubin with bromine and iodine, and have arrived at the conclusion that the resulting substance was biliverdin. Thus Maly (see Gmelin's *Chemistry*, Cavendish Soc. edit., vol. 18, p. 74) stated, that the conversion of bilirubin into biliverdin may be effected by bromine or iodine, by acids with access of air, by alkalies with access of air, more quickly by peroxyde of lead. In order to appreciate these statements, it must be borne in mind that with the exception of the one referring to the influence of alkalies and air, none were supported by any analytical qualitative or quantitative evidence. Further, that the statements of Maly regarding the nature and relations of bilirubin and biliverdin have from time to time undergone important modifications at his own hands. Thus he considered the change which bilirubin undergoes with glacial acetic acid, as an elimination of ammonia, and believed the green substance formed to be non-nitrogenous and yet identical with biliverdin. By adding ammonia to his supposed biliverdin he again obtained bilirubin. He has himself corrected, but not explained this error. He merely dissolved his bilirubin in the acetic acid, and by heating probably acetylated a small portion, which dyed the bilirubin green. In a similar manner he has mistaken for biliverdin a number of green derivatives of bilirubin, which all differ from each other, and simply have this



in common, that they possess a green colour. With regard to the alleged formation of biliverdin from bilirubin by bromine, Maly said: 'In contact with bromine vapour and moist air, bilirubin quickly turns green. When a dilute alcoholic solution of bromine is dropped into a solution of bilirubin in chloroform, the colour changes, according to the quantity of bromine, to green, dark blue, which remains unaltered for weeks, then dirty violet, dark wine-red, and ultimately light wine-red—the same changes of colour, therefore, as those produced by nitric acid containing nitrous acid.' The change was explained as oxydation.

In contact with bromine vapour and moist air, bilirubin perhaps turns green for an instant, namely as long as the orange powder is able to send yellow rays through the blue compound, which quickly covers its surface. But often as I have repeated the experiment, it has had the same result in moist as well as dry air; never has there been formed a matter or a colour similar to biliverdin, but always the brominated products described.

Further, if the green colour produced in the chloroform solution of biliverdin by bromine had been due to biliverdin, the latter must have been precipitated, as it is insoluble in chloroform. The green colour, according to my explanation, was simply a mixture of the yellow of the original solution, with the blue of the brominated product. The dark blue when once obtained remains unaltered for weeks, a good proof of the difference of this reaction from that of Gmelin, in which the blue produced by nitrous acid is of the most transient nature. The spectroscope easily shows that the two blues are due to entirely different chemical entities. Even the blues produced by nitrous nitric acid in different bile-colouring matters are different. Their different spectra were originally observed and described by me in 1866 and 1867, in the *Ninth and Tenth Reports of the Medical Officer of the Privy Council*. See the latter volume, pp. 251 to 260. Cholocyanin; its sulphate; sulphate of sulpho-cholocyanin given above; and hyocœrulin. Therefore in reactions with bile-colouring matters a blue colour is no more a proof of identity than a green.

A most elaborate account of the alleged oxydation-products

of bile-pigments and their absorption bands was published by A. Heynsius and J. F. F. Campbell, in *Pflüger's Arch. f. Physiol.* iv. 497-547, extending over fifty pages. A blue substance, *bilicyanin*, was obtained by what is termed the oxydation of bilirubin by bromine-water. The spectra obtained varied, as also did the solubilities of the products. Not a single product was isolated, and none was analysed. It is easy to see that these products were principally mixtures of the mono- and dibrominated bilirubin. Of oxydation there is no evidence whatever. The same remarks apply to a greenish product, obtained formerly by Stockvis, and termed *choleverdin*, which after perusal of the paper just quoted, he declared to be identical with and thenceforth termed *bilicyanin* (*Neues Repert. f. d. Pharm.* 21, 732-737). These discursive papers relate merely to experiments made with dilute impure solutions in test-tubes, and do not start with any pure substance, or arrive at any stoichiometrical conclusion.

My researches on this subject had proceeded thus far, when a paper was presented to and printed by the Imperial Academy of Sciences of Vienna, which, based as it was upon a plagiarism committed upon a private letter, compelled me to address to the Academy a remonstrance in the shape of an open letter, of which the following is an abstract. This letter was published in *Pflüger's Archiv*, *Liebig's Annalen*, and in the *Chemical News*, under the title of 'Open Letter to the Imperial Academy of Sciences at Vienna, containing an Examination of the Researches on the Colouring Matter of Bile, by Richard Maly, of Graz.'

The 72nd volume of the *Proceedings of the Imperial Academy of Sciences* (Part 3, October 1875) contains a paper by Richard Maly, of Graz, entitled 'On the Action of Bromine upon Bilirubin,' which compels me to communicate to the Imperial Academy the following statement:—

The paper by Prof. Maly alluded to is described as the fifth of a series, the first and second members of which are printed in the 57th and 59th volumes of the *Proceedings* of the Academy: the third paper is contained in *Liebig's Annalen* (vol. clxiii.); the fourth, again, in the *Proceedings* of the Academy (vol. lxx.). I am compelled to refer to these four

previous papers by the contents of the fifth in the 72nd volume of the *Proceedings* of the Academy.

In the introduction to the fifth paper Prof. Maly states that, through some experiments, undertaken with the view of learning something about the blue products of oxydation which nitric acid produces with bilirubin, he 'has been led to the study and discovery of a body which was totally different from the one he had proposed to himself.' Incidentally he remarks that his experiments are the fruit of a year's labour.

Prof. Maly next recants the opinion which he had maintained in all his former papers, and which in the second paper he had endeavoured to prove by many and laborious volumetric experiments, according to which the products of the action of bromine upon bilirubin owe their origin to a process of oxydation; and he then describes, after many remarks upon the alleged striking similarities between the action of nitric acid and that of bromine upon bilirubin, his great surprise on finding that the products of the action of bromine upon bilirubin are by no means oxydised, but contain bromine in substitution for hydrogen.

In a footnote Prof. Maly then states:—'After I had concluded the following research, and drafted the manuscript, I became acquainted with a research by L. W. Thudichum, "Further Researches on Bilirubin and its Compounds," which is contained in the lately-published (May) number of the *Journal of the Chemical Society*. I have learned from this that Thudichum also has recognised that the action of bromine upon bilirubin produces *bromo-products*; therefore this observation, which has also been made by Thudichum, is in any case, on account of prior publication, to be considered as his property.'

I interrupt the quotation of the footnote in order to point out that Prof. Maly represents to the Academy that he had discovered the substitution by bromine, of hydrogen in bilirubin, by means of his own experiments; that he had himself independently found out and corrected his former errors, which he had maintained for years, and which have been repeated in many chemical publications; and particularly that he had only

obtained knowledge of my researches after he had completed his research and written out the draft of his paper.

However, on June 11, 1874, I had directed a letter to Prof. Maly, in the course of which I had made to him the following communication, given in the words of the copy which I preserved:—‘I was pleased that as regards biliverdin you now agree with me. You double the formula, but without giving any reason for it. However, in the course of time our agreement will, no doubt, become greater yet. You still believe, *par exemple*, that the products of the action of bromine upon bilirubin are products of oxydation, whereas I have shown already two years ago, that they are products of substitution. Simple addition of bromine vapour to bilirubin gives  $C_9H_7Br_2NO_2$ , and determines the atomic weight of bilirubin accurately at 163, the same number which results from all my other researches.’

That Prof. Maly had received the letter containing the foregoing passages is proved by the reply which he addressed to me, dated from Innsbruck, June 14 (1874), now before me. Prof. Maly, therefore, before he began the experiments which are so exhaustively described in the fifth paper, was not only informed of his error, but actually in possession of the key to his alleged discovery, and it was therefore impossible that he should have been led to this discovery by his experiments.

It is further quite clear, from the connection of the statement of Prof. Maly, that if he had not obtained information of my paper in the *Journal of the Chemical Society*, he would in that case also have ignored my letter to him, and would have claimed the priority of the discovery of the brominated product of bilirubin. For although, in the beginning of his fifth paper, he declares, as he expresses himself, ‘the characterisation of the hitherto existing knowledge concerning the blue product of bilirubin’ to be a ‘usual duty,’ yet in enumerating previous data he neither mentions my letter nor the fact that I had published the existence of the bromo substitution-product of bilirubin already in the year 1872, in my *Manual of Chemical Physiology* (London, 1872, p. 72), and had communicated this observation to the Chemical Section of the German Association for the Advancement of Science, at the meeting at Wiesbaden.



He is perfectly silent regarding the numerous blue and variously coloured derivatives of the colouring matters of gallstones, which I was the first to characterise from my own researches, chemically as well as spectroscopically, in my Report in the *Ninth* and *Tenth Reports of the Medical Officer of the Privy Council* (1866 and 1867, pp. 251 to 260), and in the Manual alluded to. That these researches and publications should have remained unknown to the editor of an annual report on the progress of animal chemistry is not impossible, but that he excluded the contents of my letter from the circumference of the 'usual duty' admits of only *one* explanation, but not of justification.

In the note alluded to Prof. Maly says—'Thudichum has not analysed his body, but has only drawn a conclusion concerning its composition from the increase in weight which bilirubin undergoes when bromine vapour is passed over it. When bromine was passed for a short time Thudichum obtained a body which was soluble in alcohol with almost monochromatic blue colour, and contained 35·30 per cent. Br; and this, in accordance with Thudichum's formula for bilirubin, is said to be monobromo-bilirubin, for which, however, calculation requires 33·0 per cent. Br. This substance, from its properties, may correspond to the body which I describe in this paper, but without being approximately pure.'

These statements are exclusively taken from the preliminaries of my paper, which I relate only because they lead to and necessitate the performance of my cardinal experiment. The product containing 35·30 per cent. Br I have never declared to be monobromo-bilirubin; on the contrary, I have stated that by solution in concentrated sulphuric acid, and precipitation with water, to which it had been subjected, it had acquired different properties—among others, a green colour. I say expressly that the product before treatment with sulphuric acid appeared to have been a mixture of mono- and dibromo-bilirubin. I then assert that these bodies cannot easily be separated from each other by ordinary solvents.

Prof. Maly says further in the note—'When bromine is passed for a long time (over bilirubin), then, according to Thudichum, a body is produced which is yet richer in bromine,

and this he claims to be bromo-bilirubin.' And further in the text—'It is therefore' (namely because according to Maly the proper point of bromination can only be discovered with the aid of solvents) 'impossible to draw any conclusion regarding the composition of the products of the two experiments of Thudichum, in which bilirubin was exposed for some time to bromine vapour, and then weighed, for in this form the body is always fused like a resin.'

This statement of Prof. Maly is not only contrary to my actual description, but is directly opposed to the stated motives which led to my experiment. For I say that it was evident, from the preliminary experiments, that bilirubin in the presence of hydrobromic acid and moisture could not be completely brominated, just because it deliquesced, and that for this reason I had instituted an experiment in which this action was completely avoided.

I then describe the experiments with bromine given in the substance of my researches and which need not here be repeated.

I further describe the properties of the new compound, and particularly that it becomes quickly changed in several solvents. It would have been more to the interest of Prof. Maly if he had studied my statements relating to these changes, and had repeated the experiments,—if he had heeded my warning, and excluded moisture and hydrobromic acid from his experiments; it would have saved him much disappointment had he comprehended that I saturated bilirubin with bromine by offering it an excess, and did not only, as Maly reports, pass it over for some time. All these necessary precautions Prof. Maly has neglected, and in consequence has arrived at conclusions which have no foundation.

Prof. Maly further endeavours to influence the judgment of the Academy by raising doubts in general regarding my experiments; first, on the ground that I had performed each experiment only once; secondly, because I had not analysed the final product. In order to meet this objection, I have repeated the experiment described yet two several times, and have analysed the products by determining quantitatively the amounts of carbon, hydrogen, nitrogen, and bromine; during the elementary combustion none of the hypothetical bromide of carbon

was observed, with the alleged escape of which Prof. Maly endeavours to explain his discordant carbon-numbers.

In two paragraphs the experiments and analyses here alluded to are described in detail; as they are given in the text of my rescarches, they have not here been repeated.

In his remarks on the formula assumed for his bromine product, Prof. Maly says—‘The most simple expression of the composition of bilirubin is, according to the analyses of Prof. Städeler and myself,  $C_{16}H_{18}N_2O_3$ .’ In making this statement Prof. Maly loses sight of ‘the usual duty of characterising previous knowledge,’ or other knowledge. I therefore felt myself called upon to search for the analyses of Prof. Maly, of which he claims that they prove the above formula, but with the exception of one carbon and two hydrogen determinations, in the *Reports of the Vienna Academy* (1868, vol. lvii. p. 97), I could not find any proofs of this assertion. In particular, Prof. Maly has not made any determinations of the nitrogen, and, above all, no determination of the atomic weight, and has not even subjected his results to control on different preparations. For these reasons I consider his formula as of little significance opposite my theory, which is based upon numerous preparations and well-defined compounds, and now more than fifty elementary analyses.

Prof. Maly, in the paper just quoted, supports his formula for bilirubin by the authority of Städeler, but on p. 102, note 2, he censures the proceedings of Städeler, who had produced a formula for biliverdin by recalculating an old analysis of Heintz, which had been executed upon material declared by Städeler himself to have been a mixture of pigments. Prof. Maly himself points out that this proceeding was characteristic of the work of Städeler. I dare not trouble the Academy with a criticism of the researches of Städeler, particularly as I have given one in my paper contained in the *Journal of the Chemical Society*, 1875. I think it, however, necessary to direct the attention of the Academy to the circumstances that the doubling of the formula  $C_{16}H_{18}N_2O_3$  into  $C_{32}H_{36}N_4O_6$ —which Prof. Maly introduces in the fifth paper, in 1875, as a necessary consequence of his discovery of a brominated compound, and then further particularly as a novelty—had already been adopted by Städeler,

before 1870, in a letter to the editor of *Gmelin's Handbuch*, Prof. Karl Kraut, and been published by the latter in an Appendix to the last volume of the *Handbuch*. In the letter alluded to, Städeler, in view of my researches, abandons all his former formulæ, and coerces my results by an utterly unjustifiable process of recalculation, in which no single analytical result harmonises with the new hypothesis into some sort of support for his doubled formula and hexabasic acid hypothesis without having produced a single compound or made a single new analysis.

Prof. Maly causes to himself many difficulties by his preconceived opinions and uncontrolled imagination, as I am obliged to prove now more in particular. At first he believed every green product of the colouring matter of gallstones to be 'biliverdin;' thus the product by chloroform and glacial acetic acid, which seduced him into the belief that he had transformed bilirubin, an alleged amide, into ammonia, on the one hand, and an acid free from nitrogen, viz. biliverdin, on the other; and, what was still more surprising, that he had retransformed this acid into the original amide, bilirubin, by simply mixing it with ammonia (see 'Preliminary Communication on the Colouring Matter of Bile'—*Reports of the Meetings of the Vienna Academy*, vol. xlix.). A part of this conclusion Prof. Maly has withdrawn (*Reports of the Vienna Acad.*, 1868, p. 98), namely the one concerning the decomposition of bilirubin into ammonia and 'biliverdin.' The ammonia which he had before found he then explained to have been an impurity of his preparation. But in 1868 he still insisted upon the erroneous proposition that the product of heating bilirubin, glacial acetic acid, and chloroform, in sealed glass tubes, was 'biliverdin.'

For a long time he also believed his brominated product to be 'biliverdin' (*Vienna Acad. Reports*, 1868, p. 104). I have shown that in its first form it was a mixture of blue brominated bilirubin with orange bilirubin: this Prof. Maly now admits himself, in his fifth paper, as his original discovery.

In the fifth paper Prof. Maly again produces 'biliverdin' by treating the brominated product with alkalis. But it contains always yet a small trace of bromine, and a little ash, which is deducted. In the analyses 3 and 4 of his former 'biliverdin'



(*Vienna Acad. Reports*, vol. lvii. p. 105) he was even obliged to deduct as much as 'circa 2 per cent.' of ash. How can an author who works with such preparations call others to account for the alleged impurity of their preparations!

In short, Prof. Maly is unacquainted with the fact that there is a great number of derivatives of the colouring matter of gallstones, which all have this in common, that they are *green*, but are not for that reason alone biliverdin ( $C_8H_9NO_2$ ). Thus the green cholothallin obtained by the action of oil of vitriol upon bilirubin, and subsequent treatment with water, is bilirubin to which the elements of water have been added.

In two paragraphs I give a summary of the data concerning the action of bromine and hydrobromic acid upon bilirubin, contained in the text of my researches.

Prof. Maly operated with bromine in moist chloroform. Every molecule of bromine yielded him a molecule of hydrobromic acid, which now in its turn attacked the bilirubin. The moisture precipitated the mixture. The precipitate consisted proximately of bromo-bilirubin, with some hydrobromic acid, of which it yet lost some on drying, and of perhaps a little hydrobromo-bilirubide, for the action of HBr requires more time than that of Br. But now the product was put in alcohol, which immediately began its reducing action. Consequently the powder was 'dark blue-green,' while the probable monobromo-bilirubin is monochromatic blue, as I state in my essay (and not 'nearly' monochromatic blue, as Prof. Maly reports my description).

Again, the ethereal solution which Prof. Maly obtained when treating bilirubin in ether with bromine was 'dark greenish-blue,' and became blue only when the monobromo-bilirubin prevailed, by means of its greater absorptive power for green. The residue of this ether process was 'always in thin layers, green.' This change from blue to green was evidently the reason which caused Prof. Maly to abstain from further attempts at purifying his products. The incessant loss of bromine which the products of substitution, as well as of reduction, experience in alcohol or ether, is so great and marked that in some of my experiments the amount of bromine fell from 49.8 to 16 per cent., and the violet product, which had been soluble

in ether with a violet colour, became green, quite insoluble in ether, but soluble in alcohol with a green colour.

After the foregoing I hardly know whether there is any part of Prof. Maly's research left which requires refutation or explanation, excepting perhaps the remarkable results of his carbon determinations in which the carbon found varied between 35.51 and 47.83 per cent. However, this is by no means incumbent upon me, but upon Prof. Maly. The assumption of 'a very volatile bromide of carbon' cannot satisfy the demand for explanation,—much less can the proposition that an accurate determination of the quantity of carbon was not essential to the ascertaining of the composition of the bromine product. On the contrary, it must be maintained that such results and corollaries are directly opposed to the principles of chemical science, and slap the endeavour for final accuracy rudely upon the face.

We have seen above how Prof. Maly believed every green product of the metamorphosis of the colouring matter of gallstones to be 'biliverdin.' In the same manner he assumed every blue product to be a result of oxydation, and identical with the blue product which forms transiently during the reaction of bilirubin with nitric containing nitrous acid. But the blue product which is obtained with bromine is now transmuted into a product of substitution, and there is no reason to believe that the blue product obtained by means of nitrous acid is a product of oxydation. Indeed it is impossible to predict what it is. Pure nitrous acid forms no blue product with bilirubin. It seems that the presence of alcohol is necessary for the production of a blue body. From my experiments, it follows, first, that the formula which Prof. Maly has given for his 'eholoteline' is improbable, and that the diminution of the carbon in this reaction which Prof. Maly observed—but left out of consideration or explained away—is much more probable than the alleged oxydation without any loss of carbon, particularly as the nitrogen in the product is the same quantity as that which was present in the bilirubin employed. The three silver compounds of eholoteline, in which the silver rose in quantity from the first to the third, of which, however, only the second one furnished an acceptable theory (*Vienna Acad. Rep.* vol. lix. p. 605), are

analogous to the zinc compounds of hydro-bilirubin, in which the zinc rose from 14.6 to above 37 per cent. (*Liebig's Ann.* clxiii. 86).

The observation of the influence of sodium-amalgam upon bilirubin, which led Prof. Maly to the discovery of the so-called hydro-bilirubin, would have been an interesting progress in our knowledge concerning bilirubin. But as the author starts from erroneous views regarding the composition and molecular weight of bilirubin, his conclusions regarding his product and its composition, and regarding the formula of the change, are necessarily erroneous.

The announcement at the head of this article on hydro-bilirubin, of the transformation of bilirubin into the colouring matter of urine caused me to read it with expectations which were speedily disappointed when the absolute 'urine-colouring matter' became limited to Jaffé's urobilin. I thereupon made many experiments which I have described in my paper (in the *Journ. Chem. Soc.* May 1875), and which, without exception, negative the alleged metamorphosis. On this point I wrote to Prof. Maly:—'I have now compared the products (of the reduction of bilirubin) with all urinary colouring matters with which I am acquainted, and with Jaffé's product, which I have produced for the purpose, but have not discovered any identity. Urochrome and uroerythrin are quite different as regards solubility and chemical properties; uroxanthin, which it is now the fashion to call "indican," also. The products of the cleavage of urochrome—namely uromelanin, uropittin, and omicholin also. These products can be easily obtained from urochrome by acids, but your hydro-bilirubin yields nothing of the kind, and is not much changed by boiling with hydrochloric acid. Jaffé's urobilin has never been isolated, never been analysed. According to my comparison the mass is a mixture of urochrome, uroerythrin, with a little omicholin already separated. The process (of Jaffé) is not inviting, and the result in any case, a complicated tincture. I am therefore unable to adopt your conception of the metamorphosis of the colouring matter of bile, into *the* or *a* colouring matter of the urine in even a single particular.'

In his fourth paper (*Vienna Acad. Rep.* vol. lxx. 1874) on biliverdin, Prof. Maly relates how he 'already earlier and inde-

pendently of that of Thudichum,' had arrived at the formula  $C_{16}H_{18}N_2O_4$ . However I had only arrived at half that formula, namely  $C_8H_9NO_2$ ; but this difference is unimportant; it is quite true that I arrived at this formula independently of Prof. Maly, but I have also a priority with reference to it which does not appear quite clearly in Prof. Maly's representation; for my research on biliverdin was communicated to the Royal Society already on November 14, 1867, and published in abstract in the *Proceedings* of that Society, vol. xvi. p. 217. The relative research of Prof. Maly on the other hand was communicated to the Academy of Vienna only on February 6, 1868.

In the paper No. IV. just alluded to, concerning biliverdin, Prof. Maly communicates two further elementary analyses of biliverdin, and, as now his results exactly correspond with mine from the year 1867, he is satisfied that the composition of this body can now be considered as definitely established. This time the biliverdin did not contain any ash, and was made according to the orthodox method (of Heintz), with soda and air. There still figures the biliverdin made by means of chloroform and glacial acetic acid, but it cannot easily be precipitated by water. A new green matter obtained by monochloroacetic acid from bilirubin, also makes its first appearance. The metamorphosis each time yields less 'biliverdin' than the weight of the employed bilirubin, but was obliged in each case to support the theory without analysis.

But now comes the buttress of Prof. Maly's theory, according to which biliverdin is bilirubin plus oxygen only,  $C_{16}H_{18}N_2O_3 + O = C_{16}H_{18}N_2O_4$ . Bilirubin is transformed into biliverdin, according to the orthodox method, with soda and air, and the product weighed; 0.4558 grm. bilirubin yields actually 0.4458 grm. biliverdin, both dried at 100°. A loss was therefore sustained. There was no guarantee that the biliverdin did not yet contain bilirubin. But the hypothesis demanded a greater weight of biliverdin than was that of the bilirubin employed. In consequence the filtrates were now evaporated, and what they lost by ignition was scored as biliverdin. Even the washing water is put into requisition and compelled by means of a process termed 'ocular measurement' (*Augenmessung*) to supply more than half of the desired increase. Thus by hook



or by crook Prof. Maly succeeds in calculating an increase of 4·3 parts upon 100 of bilirubin, whereas his hypothesis demands an increase of 5·6 parts. ‘This,’ says Prof. Maly, ‘agrees as accurately as can be demanded under such circumstances.’

As often as the experiments agree very badly with the hypotheses of Prof. Maly, and prove rather the contrary than the hypothesis, he consoles himself with the expression that the result was as good as could be demanded under the circumstances. Thus again, in his first synthetical bromination experiment, paper V., he finds an addition of only 2·74 atoms of bromine to his new bilirubin,  $C_{32}H_{36}N_4O_6$ , but observes—‘This accorded so far with the increase by 3 atoms of bromine which were demanded by calculation and supported by experiment, as could be demanded in determinations of this kind.

But the very next synthesis yields 3·1 atoms of Br; the experiment could therefore be made in a more accurate manner than could previously be demanded. But this experiment also has no value at all. Bilirubin, treated with bromine in excess, yields, as I have proved, always dibromo-bilirubin,  $C_9H_7Br_2NO_2$ , in which  $N : Br = 1 : 2$ . In Maly’s supposed molecule  $N : Br = 4 : 3$ . Consequently if my product were quadrupled, in order to let it contain 4 N, it would contain 8 Br,—say 8 atoms of bromine. In any case, therefore, Maly would only have introduced 3 atoms of bromine out of 8 which can be introduced. But this high formula is so entirely negatived by my compounds of bilirubin with silver, calcium, baryum, lead, zinc, and others, that it cannot come into consideration.

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#### ERRONEOUS STATEMENTS OF HOPPE-SEYLER CONCERNING THE COLOURING MATTERS OF BILE.

After my research had been published (*Journ. f. pract. Chem.* 104, 1868, 1) Hoppe-Seyler wrote a notice of it in the *Medical Jahresbericht*, which had more the character of an aspersion than that of a report, or even of a criticism. He himself had no personal knowledge whatever of these substances, and has up to the present time not published any researches

upon them. He had therefore not the slightest qualification to as much as doubt my researches, and the challenge which I addressed to him as well as to the editor-in-chief of the *Jahresbericht*, Prof. Virchow, that he, Hoppe-Seyler, should refute my researches by experiments and analyses, has to this day remained unanswered.

On the other hand Hoppe-Seyler has, in his *Handbook of Physiol. and Pathol. Analysis*, given a representation of the biliary pigments, of which the statements which Städeler made in 1863, form the sandy foundation, in perfect disregard of the fact, that Städeler himself had abandoned these, his second variety of formulæ, already before 1870. Now as Hoppe-Seyler produces no reasons why this resignation of Städeler's should not be adopted by others, and brings no new grounds for retaining Städeler's formulæ, his proceeding does not only manifest a want of acquaintance with the subject, but also, and particularly, a want of acquaintance with its literature.

Of my results Hoppe-Seyler now says, that 'it would lead too far' to describe them. This indefinite utterance is a feeble excuse for the omission of a well-recognised literary duty, and it seems to be felt when further on Hoppe-Seyler endeavours to justify his reticence by a surmise (p. 211) to this effect, that the bilirubin examined by me had been a different body from that examined by Städeler and Maly. This view was not for an instant conceived by Städeler himself, and has never been put forth by Maly. Städeler accepted my bodies and my facts, but used them, by means of an arithmetical operation, which was more desperate than reasonable, for the production of an hypothesis, intended to save his double formulæ. The assumption of 'different kinds of bilirubin' has therefore no better foundation than the imagination of Hoppe-Seyler.

Towards the end of his description of the matters in question, the confusion of Hoppe-Seyler rises to a wild conflict of errors. A colouring matter, said to form in ox-bile on standing, and formed in other ways not described, also contained in sheep's-bile, is said to be identical with the product of oxydation of bilirubin, biliverdin, and bilifuscin, by nitric acid or bromine water. It is confounded with the choleverdin of Stockvis, and the bilicyanin of Heynsius and Campbell, and it is claimed that

he, Hoppe-Seyler, had described 'the spectral phenomena' already in the earlier edition of his handbook, and that Jaffé and Bogomoloff had described them after him. In short, the entire representation of this subject given by Hoppe-Seyler is unworthy of literature and of science.

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The other protests referred to in the Preface are the following:—

'On Acetic Acid, Formic Acid, and supposed Sulphurous Acid and Nitrous Acid from Human Urine.' *Archiv Physiol.* 15, 1877, 12.

'Repetition of the Experiment of Gscheidlen for the Extraction of Sulphocyanic Acid from Human Urine.' *Archiv Physiol.* 15, 1877, 51.

'On Indican, and the relations of Prof. Max Jaffé to the definition of Chemical Purity.' *Archiv Physiol.* 15, 1877, 343.

A more general view of these objectionable practices has been taken by C. T. Kingzett and H. W. Hake, in their important article entitled 'Physiology and its Chemistry at home and abroad' in the *Quarterly Journal of Science*, 1877.





# SOME DEFINITIONS OF NAMES AND SYNONYMS.

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*Amylonide*: a body similar to amydon, or containing an amydon-like radicle in its constitution; this radicle appears as a kind of sugar after decomposition of the body.

*Cellulin*: the substance forming the cells of plants, wood, etc. The ending *in* has been substituted for the former terminal *ose*, as it seems convenient to reserve this latter for sugars only.

*Chemolysis*: decomposition of organic compounds into more simple substances by merely chemical agents, such as sulphuric acid or baryta.

*Colloid*: a body like gelatin, which does not dialyse through parchment paper.

*Crystalloid*: a body which behaves like a crystallising substance in this, that it dialyses through parchment paper.

*Fell*, to: verb; Saxon equivalent of the Latin to precipitate.

*Glycin*: the same as *glycosin* or *glykocoll*, amido-acetic acid; it is proposed to retain glycin only, and discontinue the second and third name.

*Hydrochlor*: abbreviation of hydrochloric acid.

*Hydrothion*: abbreviation of hydrosulphuric acid, or sulphuretted hydrogen; used by Bergmann and Berzelius, and by Gmelin throughout his great treatise on Chemistry.

*Molecule*: English form of the Latin 'molecula,' ordinarily spelt in the French form 'molecule.'

*Organoplastic*: adjective used in physiology to describe the chemical compounds which constitute tissues or organs; tissue-forming or histogenetic would be the same, but less euphonious.

*Patholysis*: decomposition of organic substances in the living body under the influence of disease-ferments, or disease-causes in general.

*Physiolysis*: decomposition of organic substances under the influence of water, air, and putrefaction-ferments; therefore equivalent to putrefaction, but wider in its significance.

*Pyrolysis*: decomposition of organic substances under the influence of a raised temperature only; dry distillation; a process compared to the burning of a body in the presence of that oxygen which is contained in it.

*Qualitation*: process of finding out the quality, therefore abbreviation for qualitative analysis.

*Quantation*: process of ascertaining the quantity, therefore abbreviation for quantitative analysis or estimation, or determination.

*Radicle*: English form of the French 'radical,' analogous to 'molecule.'

*Sarcolactic acid*: lactic acid from flesh, formerly termed paralactic acid.

*Zymolactic acid*: lactic acid obtained by fermentation, *e.g.* of milk, or sugar with cheese.

*Zymolysis*: decomposition of organic substances by ferments, organised or shapeless.

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